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Melanoblasts and Melanocytes
in Fetal Negro Skin

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Contents

I. Introduction

During the past decade, important changes have occurred in the basic concepts of the origin and functional relationships of mammalian and human cutaneous pigment cells. Twenty to 30 years ago, the dominating view was based mainly on Bloch's theory that ordinary basal cells of the epidermis were the pigment producers under appropriate stimuli, and that any true pigment cells encountered in the dermis could have attained their location only by a descent or *Abtropfung* either in early or later developmental periods.

Pigment formation in man and other mammals thus was placed in a separate category. The essential biological events were said to be different from those known to occur in other vertebrates. That theory failed to account for pigmentary conditions in certain primates and rodents in which the dermis is heavily pigmented and the overlying epidermis remains practically free of pigment cells. Adherents to Bloch's theses were inclined or compelled to consider certain normally occurring pigmentary features, such as the so-called Mongolian spots, either as histological curiosities or as having developed from the embryonic epiblast (somatic ectoderm) at a very early period. No evidence had ever been given for the latter supposition.

The newer and most widely accepted views deny that the skin-ectoderm has any pigment-forming potentiality. It has long been known that in other vertebrates, especially amphibians and birds, the source material for the cutaneous pigment cells resides in the neural crests. These structures constitute paired strands of cells that develop as neurectodermal derivatives on the dorsal sides of the closing neural tube, very early in embryonic life. They are of an evanescent, transitory nature, never existing through the full length of an embryo, but differentiating into several types of cells of which the future cutaneous pigment cell is only one. The potentialities of the neural crest are indeed impressive.

The credit for experimentally proving that in mammals, too, the pigment cells are derived from the neural crest belongs to Rawles (1947, 1948). Her ingenious grafting experiments showed conclusively that in order to produce pigment, the mammalian epidermis or its hair follicles are wholly dependent on a migratory cell that enters the somatic ectoderm secondarily. Rawles never actually identified the migratory forms of the later pigment cells.

Zimmermann and Cornbleet (1948) observed the first potential pig-

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ment cells of the fetal Negro epidermis early in the third month. They are not modified basal cells, which begin to reveal melanin granules late in the fifth or in the sixth months — long after the true pigment producers or melanocytes begin to elaborate melanin. But no one had yet shown that the prenatal human dermis contains any migratory cell stages of future melanocytes.

If the derivation of pigment cells from the neural crest was to hold true also for man it obviously became a primary, logical, and challenging task to attempt the identification of their precursor stages in the dermis. This report deals in part with the differentiation of melanoblasts in the dermis, beginning at 10 and 11 fetal weeks, to melanocytes in the dermis and epidermis. On the basis of extensive material, consisting exclusively of Negro fetuses, we were able to study a pigmentary cell type that hitherto had not been demonstrated to exist throughout the dermis in the human prenatal period.

The development of distribution patterns and population densities of pigment-producing cells in the fetal epidermis was also followed in greater detail and with more reliable methods than had been applied before. Certain corrections of published data were made. A strictly statistical treatment of the numerical data, however, was not intended. Instead, we deliberately placed the emphasis on new and detailed evidence for the concept that pigment cells invade the epidermis secondarily. We therefore established their time of arrival at ultimate destinations in various cutaneous regions. This naturally led to cell counts. In the early fetal period, the migration and arrival of melanocytes at the dermo-epidermal junction was found to be irregular. The variability in cell counts of melanoblasts and immature melanocytes in the dermis of the third and fourth fetal months reflects their migration in scattered groups or "swarms." This, in turn, affects the earliest distribution patterns and population densities of melanocytes in the epidermis. A reliable biometrical evaluation of such data appeared to be impossible. The pertinent conditions in the most interesting developmental period were found to be in a highly fluid state (see legend to Table 1).

Since a standardized nomenclature for cellular elements concerned with pigmentation is not yet established, we define our terms: A "melanoblast" is strictly an embryonic type of cell, potentially capable of producing melanin but not containing the fully elaborated pigment. Melanoblasts exist only in the dermis and are first detectable early in the third fetal month. Their round or ovoid cell bodies contain granules that have a specific affinity for reduced silver but cannot be impregnated with ordinary silver nitrate. This is generally accepted to mean that such cells contain a form of "premelanin." Melanin is capable of reducing silver nitrate directly.

The melanoblasts, recognized by us from the tenth to twelfth weeks of human development, should be considered as the end stages of a chain of precursor cells that are derived from the neural crest. The earliest migratory forms in that chain are undistinguishable from ordinary mesenchymal cells. Since the neural crest of man develops in a cephalo-caudal direction during the second embryonic month, a gap of several weeks remains in the demonstration of the ultimate origin of pigment-producing cells. That gap has been filled by experimentation with mammals (Rawles). There is no reason to doubt that the events are the same in man as they were proved to be in mouse embryos. Certainly there is no evidence to the contrary.

Melanoblasts rapidly develop one to three short, stubby extensions, the precursors of the future dendritic processes. Thereby they begin to differentiate into *melanocytes*. Various transitional forms of fusiform and young stellate cells, containing increasing amounts of premelanin granules, make it difficult sharply to distinguish between melanoblasts and melanocytes. Our definition of the former is definitely more restrictive than that implied by the original term "melanoblast" as introduced by Ehrmann (1885).

Whether melanocytes begin to elaborate melanin while still in the dermis cannot be used as a criterion for a valid definition. In white skin, for example, the melanin-producing tyrosinase system of melanocytes is generally or temporarily inhibited, except in the sacral region (Mongolian spots). In conditions of vitiligo, the suppression of pigmentary activity is even more pronounced and it is well known that in albinism there is a total lack of the enzyme, although the melanocytes are present. Billingham (1948, 1949) and Billingham and Medawar (1953) designated such inhibited cells of white skin as "white" or "non-pigmentary" melanocytes. Obviously, a broad definition of melanocytes must rely on purely morphological features. Whether immature or fully differentiated, they usually have fusiform or stellate cell bodies and several processes. Their potential melanin-producing faculty may or may not become apparent.

All melanocytes are temporarily in the dermis. Some remain there until birth, or, as in the sacral region, until later childhood. The great majority, however, become *epidermal melanocytes* by an active invasion of the epidermis. In the Negro, this process begins in the eleventh and twelfth weeks of intrauterine development. While still in the dermis, the fusiform or stellate cells may be designated as immature "dermal" melanocytes. This working term, of course, is not meant to imply in any way that such cells are of dermal (mesodermal) origin, any more than the epidermal melanocytes would represent a modified epidermal cell. The adjectives "dermal" and "epidermal" are merely used for the sake of

brevity and indicate location as one of the major characteristics of the cells concerned.

II. Literature

The vast literature on problems and concepts of cutaneous pigmentation in man contains relatively few contributions that deal specifically with prenatal conditions. We limit our review to studies that concern (a) the histological identification of pigment cells in the pre- and postnatal dermis of man; (b) similar comparative data in other mammals, particularly in primates; (c) the recognition of prenatal melanocytes in the human epidermis; (d) data on population densities and gradients of distribution of such cells; and (e) the experimental evidence for the origin of mammalian melanocytes from the neural crest.

(a) *Pigment cells in the pre- and postnatal dermis of man.* The presence of such cells was first established by Baelz (1885), who identified them as early as the fifth fetal month in the sacro-coccygeal region of a Japanese fetus. Baelz considered the macroscopically visible pigmentation of that area as an important characteristic of the Mongolian race. Henceforth these pigmented, bluish areas became known as the so-called Mongolian spots. Grossly, they had been noticed by Japanese medico-popular writers for some hundred years before Baelz's study, and many superstitious notions had been attached to them. Baelz noted also that similar pigmented cells had characteristic relationships to hair follicles, in which they formed a "regular network" immediately above the papilla.

The first mention in modern science of blue sacral skin areas was by Eschricht (1849), who had obtained second-hand information of their occurrence in a full-term Eskimo fetus.

An important study of pigment cells in the normal corium of Japanese fetuses, newborns, and children was made by Grimm (1895). Grossly, he found the blue spots in the sacral region of all newborn Japanese and noticed that the pigmentation in the spots increased during the first postnatal months and began to fade in the second year. In the dermis of the sacral region Grimm detected the earliest pigment cells in the third and fourth fetal months. In the Mongolian spot of newborns they were large, plump, or elongated fusiform cells, sometimes provided with several processes. The processes often connected with those of similar neighboring cells, and whole "trains" were thus interconnected. Grimm emphasized the irregular distribution of these cells, often in dense "swarms," located in the deeper two-thirds of the dermis, never in the papillary zone. Their length varied between 20 and 50 μ.

Earliest cell forms of the third and fourth fetal months were round or

oval, coarsely granular. Some measured 15 μ in the long axis and 7 μ in width. Grimm conjectured that they originate in very young embryos. He found no evidence for their derivation from connective tissue cells, as had been postulated, and cautiously left the question of their origin open for further embryological and comparative studies.

Grimm found that dermal pigment cells did not invade any epidermal derivatives (sudoriferous and sebaceous glands, root sheaths of hair follicles). Although he confirmed Baelz's observation on the arrangement of pigment cells in hair papillae, he could not prove that they were directly derived from the dermis. Grimm's study contains no illustrations of microscopic sections but shows the gross appearance of the blue spots in Japanese children.

Adachi (1903) was the first to identify pigment cells in the dermis of the sacral region in infants and children of the white race. He thereby disproved the opinion that blue spots in the sacral region are characteristic of a specific (Asiatic) race. The development of pigment in the corium of localized (sacral) areas was recognized as a normal occurrence during the later developmental stages of man in general.

Adachi studied sacral skin specimens of 76 white individuals, ranging from the fetal period to old age. He made freehand sections of alcohol-fixed material and obtained satisfactory results with unstained preparations. He could not detect any blue spots in fetuses of Europeans and was unable to identify dermal pigment cells in white fetuses or in white newborns. The earliest spots were grossly recognized in one- to three-day-old white infants and dermal pigment cells were numerous in the sacral regions of a six-month-old infant and of a 20-month-old child. Adachi described the pigmented cells in the deeper dermis as fusiform or stellate, measuring 40–80 μ in length and 4–10 μ in thickness. Some cells attained a length of 130 μ. He considered them as connective tissue elements and believed that the pigmented cells of the dermis could not reach or penetrate the epidermis. He was puzzled by a constantly pigment-free (papillary) zone between the epidermis and the pigmented layer of the dermis.

Adachi distinguished two types of connective tissue pigment cells: (a) small cells in the upper layer of the dermis, which obviously correspond to the macrophages of modern interpretation, and (b) large cells in the deeper layer. The latter unquestionably correspond to our melanocytes of the dermis and represent the crucial elements of his study.

Besides "dermal" pigment cells, Adachi described epidermal "chromatophores" that are similar in most respects to the dendritic cells or epidermal melanocytes. He believed that they were artifacts produced by the arrangement of intercellular pigment granules. In holding this view, Adachi evidently was influenced by Schwalbe, in whose laboratory he

worked in Strassburg and who, with Unna, Cohn, Rabl, and Kromeyer, was one of the protagonists of the theory that epidermal "chromatophores" are not cells at all. Ranvier, Ehrmann, Riehl, and others held, instead, that the epidermal pigment cells ("chromatophores") were fixed connective tissue cells that had penetrated the epidermis and existed in it independently. Ehrmann had introduced the term "melanoblast," refuting that it represented either a modified leucocyte, connective tissue cell, or an epidermal cell. According to Ehrmann's original definition, "melanoblasts" were derived from the early embryonic mesodermal layer.

Adachi refuted the view that the Mongolian spot is an atavistic occurrence. Since it is found in many colored races and since European children, too, have pigment cells in the corium, the potential blue spot or *Kinderfleck* had never been lost in the evolution of human pigmentary conditions. Bloch (1901), in a highly speculative report unsupported by evidence, had maintained that "the spot is a sort of rudimentary organ which gives clues for the skin color of the ancestors of the yellow race and which one may call a stigma of atavism."

Adachi held, instead, that blue spots are of a rudimentary, regressive character and that this type of pigmentation occurs in all races during a certain period of development. His comparative anatomical findings on dermal and epidermal pigmentation in primates are reviewed under a separate heading.

Kato (1905) studied Mongolian spots grossly in 600 Japanese children from birth to 13 years. He gathered statistical data on their localization, form, size, color, and rate of disappearance. The spots were also studied histologically. He recognized the earliest pigment cells in the dermis of an eight-month fetus. In some skin specimens of children he found pigment cells often adjacent to blood vessels. The cells of a deep blue Mongolian spot in a nine-month-old child were round, oval, or fusiform, and of a brown color. Many branched cells appeared connected with each other, and though the papillary zone was mostly free of pigment cells, Kato observed them occasionally at the dermo-epidermal junction. He made one of the first histological studies of a blue nevus (adult Japanese) and identified fusiform, branched, or serpentine cells of brown color in the deeper two-thirds of the cutis vera. He considered the characteristic pigment cells in the dermis of the sacral areas of children and of the blue nevus in adults as identical. Grimm had expressed a similar opinion without giving histological evidence.

The gradual disappearance of the Mongolian spots in later childhood was explained by the disintegration of pigment cells and the absorption of pigment granules by dermal lymphatics and venules. In the blue nevus of adults the cells were said to remain functional and to retain their characteristic form.

Bloch (1921) also attempted to find the site and time of the earliest pigment formation in white fetuses. His material consisted of 12 specimens ranging from the third to the ninth month. Pigmentary elements within the dermis were encountered in the sacral region of a fetus of about five months. Bloch described them as "peculiar cells which are not related to the normal pigmentation of the epidermis." These dopa-positive melanocytes in the dermis remained "a puzzle as to their origin and significance." They were the only exception to Bloch's concept of the localization of pigment production in strictly epithelial cells of ectodermal origin. Since he detected them only in the sacral area (Mongolian spot), Bloch designated them as *Mongolenzellen*. Nonetheless, he was aware that a complete answer could not be expected from his limited material.

El Bahrawy (1922) studied under Bloch and tabulated the published reports of Mongolian spots according to their geographical and racial distribution. They were seen macroscopically in newborn and children: 100 per cent in Japanese and other Mongolians, 80 per cent in Negroes and 2 to 4 per cent in Europeans. In white children without visible sacral spots the pigment cells in the dermis were pale, scarce, and easily missed. He made histological observations on sacral skin of 112 European cadavers (unstained, stained sections, and silver impregnations according to Bizzozzero). Pigment cells in the sacral dermis were first identified in a few white fetuses of four to five months and in all specimens from five fetal months to nine-year-old children. In specimens from 12 to 82 years of age, dermal pigment cells were seen in only four, none over 21 years old. The irregular distribution of these cells was noted in many preparations. Oval, pear-shaped, fusiform, or irregularly wavy cells measured 5–10 by 30–50 μ.

The argentaffin cells of the dermis were shown to be dopa positive and were recognized, therefore, as true pigment producers or "melanoblasts." Their normal occurrence in European children was considered as a histological or racial curiosity. Bahrawy recognized that phylogenetically such cells might represent "a temporary remnant of generalized pigmented areas in animals, where they exist throughout life."

In a biopsy specimen from the forearm of a rhesus monkey, Bahrawy found numerous fusiform pigment cells in the deeper layers of the dermis. These dopa-positive cells were believed to correspond to those of the Mongolian spot in man. The typical cells of a blue nevus and the "Mongolian cells," as well as the dermal pigment cells of certain monkeys, were thought to be of the same nature. This was an important advance in the direction of a broader concept. Bahrawy speculated, however, that the cells in question might be of early ectodermal origin and had migrated into the dermis during embryonic life. This represents an attempt to support his teacher's theory.

Ishikawa (1924) searched for the characteristic pigment cells of the sacral dermis in 32 Japanese fetuses. The earliest ones were found in two specimens (of a group of ten fetuses) of the early part of the third month. They were round or oval cells, measuring 10–15 μ, rarely 25 μ, and showing no processes. Generally they were highly scattered and contained yellowish-brown pigment granules. Similar cells were identified in the deeper layers of the sacral corium in four of eight fetuses of the fourth month. There were numerous pigment cells in the Mongolian spot areas of fetuses ranging from the fifth to the tenth month. In specimens older than six months they were mostly spindle-shaped and measured 4–10 by 30–80 μ.

Ito (1953) reported on dermal pigment cells of the Mongolian spot, the blue nevus, and the nevus fusco-coeruleus (of Ota). He stated that they were produced by "mesenchymal melanoblasts." Definite affinities with the nervous system ("suggesting endo- or perineurium") were postulated. This concept is reminiscent of Weidenreich's obsolete theory. Ito believed that during its gradual disappearance the Mongolian spot becomes estranged from the nervous system, whereas the more localized conditions of the blue nevus and nevus of Ota retain their nervous connections for life. In his studies of Mongolian spots in Japanese fetuses of varying ages, Ito reported results almost identical with those obtained by Kato and Ishikawa. As late as 1957 (second report on melanin studies) Ito still maintained that melanogenous dendritic cells were derived from peripheral nerve fibers.

Barry (1952) was the first to report dermal pigment cells in other than the sacral region of man. Working in a French laboratory at Hanoi, Indochina, he studied fetuses of the yellow race and some scalp specimens of newborn and adult individuals. The earliest dermal melanocytes were identified in silver-impregnated head sections of a 5 cm fetus. We calculated its age to be almost three months. The cells contained premelanin; some had short processes and were located in the reticulopapillary zone with their long axis lying parallel to the basement membrane. Some appeared to be migrating toward the epidermis, with processes extending to the basement membrane. There were as yet no dendritic cells in the epidermis. (French authors persistently designate the latter cell type as "Langerhans cells.") Scalp sections of a fetus of 3.5 months (8.5 cm C.R.L.) showed a few dendritic cells in the epidermis and some dermal melanocytes. In silver-stained scalp sections of a fetus of 4.8 months (14.5 cm C.R.L.) there were numerous epidermal dendritic cells and various forms of dermal melanocytes. There were also dendritic cells and dermal melanocytes in the lumbodorsal-gluteal region. The illustrations of the latter cells resemble our own photomicrographs of similar cell stages in younger Negro fetuses. At five months the dendritic melanocytes of the

epidermis were numerous in the lumbar region but the basal cells contained no pigment granules detectable with silver. There were many branched pigment cells in the dermis of the scalp, often associated with blood vessels. A similar perivascular arrangement was noted in the scalp of a newborn. The basal cells now contained fine pigment granules, seen both in unstained and silver-treated sections. No dermal melanocytes were found in the adult scalp, but the basal cells were now crowded with melanin granules capable of reducing silver nitrate.

Barry concluded that the dermal melanocytes are the forerunners of the dendritic "Langerhans cells." At first they are without processes and contain only premelanin. Later, branched forms appear, some of which he presumed to become the "dendritic melanoblasts" of hair follicles. In all races pigment granules appear in the basal cells of the epidermis long after the "Langerhans cells" have reached their destination. Barry noted that the melanocytes disappear in the dermis when the population of dendritic cells within the epidermis attains a certain density. This is the first suggestion of a puzzling "barrier" effect that seems to regulate the number of invading epidermal melanocytes per unit skin area.

Barry believed that the dermal melanocytes were derived "probably from the ectomesoderm," without specifically mentioning the neural crest and without being aware of Rawles's work. He postulated that the pigment cells of the Mongolian spot, blue nevi, hair follicles, and the dendritic cells of the epidermis originate from the same source. He strongly opposed the theory of Bloch and adduced valid evidence for his plea that it should be abandoned.

(b) *Comparative anatomical data on dermal and epidermal pigmentation in primates and other mammals.* Adachi (1903) made important contributions to our knowledge of pigmentary conditions in the skin of apes and monkeys. In primates, cutaneous pigmentation exists both in the epidermis and in the dermis (orang, chimpanzee), sometimes only in the epidermis (gibbon, spider monkey), or only in the dermis (baboon, barbary ape). There were variable amounts of melanin in either layer and the type of cutaneous pigmentation was unrelated to the animal's systematic position. In some anthropoid apes (orang and chimpanzee) Adachi observed great numbers of pigment cells in the dermis of practically all body regions. They were spindle- or star-shaped cells, measuring 80–150 by 5–10 μ. Similar cells were characteristic for the dermis of various species of Macacus (rhesus monkeys), which have a bluish skin and little epidermal pigmentation. In general the extensor side of the extremities was richer in pigmentation, in either epidermis or dermis, than the flexor side. In the chimpanzee the dermal pigment cells in the extremities often were adjacent to blood vessels.

Since in adult man the degree of epidermal pigmentation varies greatly with race, and the corium is free of large pigment cells, Adachi considered "Homo" as an independent pigmentary type.

Miescher (1922) identified similar pigment cells in certain dermal areas of the mouse. They were numerous around the vibrissae, where they formed a collarlike network at the upper end of the follicles. Blood vessels were also accompanied by dermal pigment cells, which Miescher described as threadlike, elongated, sometimes branched or star-shaped. Their dopa reaction was positive but variable. He therefore recognized them as "autochthonous pigment-producers" and likened them to the pigment cells of the chorioid of the eye. Assuming that the pigment cells in the cutis vera of the mouse, apes, and some monkeys, and those of the chorioid and of the Mongolian spot were of mesodermal origin, Miescher acknowledged that the principle of an ectodermal pigment formation could no longer be maintained or, at least, appeared questionable.

Danneel and Cleffmann (1954) showed that various species of rodents have pigmentary conditions similar to those in apes and monkeys: the dermis, the epidermis, or the hairs may be the only pigmented structures. They studied embryos and skin specimens of newborn mice, rats, and rabbits. The earliest dermal melanoblasts were identified in mouse embryos of 14 to 15 days. The cells were relatively scarce and located near the dermo-hypodermal junction. None were directly beneath the epidermis. This had been construed to mean that there is no relationship between dermal and epidermal pigmentary elements. Danneel and Cleffmann believed, however, that dermal melanoblasts rapidly ascend and penetrate the epidermis. They observed that in the ear of mice and rats such migrations occur relatively late, one to three days after birth. In the ear the dermis retains some pigment cells throughout, whereas in other body regions they disappear. On the backs of rabbits only hair follicles contain melanocytes. Danneel and Cleffmann never saw a direct migration of dermal melanoblasts into developing hair follicles. The follicles receive their pigment cells from the epidermis during development by migration along the outer root sheath. The migratory process of melanocytes stops soon after birth, and the increase in number of cells occurs by repeated cell division in the papillae. These investigators were aware of Rawles's experimental evidence and agreed with the view that all pigment-producing cells of vertebrates are derived from the neural crest.

Weissenfels (1956) contributed interesting details on the earliest phases of melanogenesis in embryos of Japanese "silky" fowl. The epidermis and feathers of adult "silkies" are devoid of pigment, but the underlying tissues contain numerous pigment-producing cells. Without giving direct evidence, the author stated that in early embryonic stages

"spindle-shaped cells migrate from the neural crest into almost all body regions." In tissue cultures he observed the origin of premelanin granules within specific cytoplasmic centers of melanoblasts. Granules were produced periodically in waves. The centers were not identical with the Golgi apparatus. Preparatory to mitosis the melanoblasts became more spherical. Some processes, crowded with granules, remained connected by slender plasma bridges. After the nuclear events of mitosis, one of the daughter nuclei usually migrated into one of the retained cell processes. No flow of pigment granules could be observed from mother to daughter melanoblasts. The latter, instead, soon began to produce their own premelanin granules from newly arising cell centers. They often formed budlike evaginations of the cell contour from which the dendritic processes arose. These events were observed both in vivo and in vitro, with the phase contrast and the electron microscope.

(e) *Data on prenatal melanocytes in the human epidermis.* Pigment-producing cells in the epidermis of Negroes before birth were discovered relatively late in the history of pigment research. The prevailing opinion had been that Negroes were born white and that pigment appeared only during the first few postnatal days, especially at the nail folds, areola of the nipples, and the external genitalia. The concept was based in part on the dicta of renowned histological authorities, e.g., Kölliker, Unna, and in part on careless examination of the hyperaemic, pink skin of the newborn.

Morison (1889) was one of the first to state that Negro children were born with some cutaneous pigment. In skin sections from the arm of an eight-month Negro fetus he observed pigment in the deepest layer of the epidermis.

Thomson (1891) identified pigment, in unstained sections of the scalp, in a five-month Negro fetus. He also observed the curved character of hair follicles as well as "interlacing pigment cells" in the hair bulbs.

Grimm (1895) had seen small amounts of pigment in the rete malpighii of Japanese newborn. Adachi (1903) corroborated this and further observed that newborn whites often had a lightly pigmented epidermis. His concept of the epidermal "chromatophore" as a non-cellular structure has been discussed above.

Bloch (1921) obtained the earliest dopa reactions in the skin of a five-month white fetus. No true pigment was detectable in either epidermis or dermis, but certain *melanoblastenartige Zellen* within the basal layer revealed a gray-brown hue. In another specimen of the same age, the matrix of hair bulbs contained "a few cells with processes that looked like 'melanoblasts' and gave a weak, positive dopa reaction." Neither the papillae nor the hair shafts contained any pigment as yet. Fully formed melanin was first identified in a few hair bulbs of the fifth fetal month and in the

epidermis proper of specimens of the sixth to seventh month. The dopa-positive cells within the basal layer were described as typical "melano-blasts" (in the sense of Ehrmann's definition); irregular, star-shaped cell bodies with branched processes. Bloch compared them with "ganglion cells of the brain cortex." He stressed again that all pigment was produced by cells of ectodermal origin (basal layer of the epidermis and hair matrix).

Zimmermann and Cornbleet (1948) recognized dendritic melanocytes within the epidermis of Negroes early in the third fetal month. They obtained positive dopa reactions, exclusively in these cells, from the fourth fetal month on. The transfer of melanin granules from melanocytes to neighboring epithelial cells was first noticed late in the fifth fetal month. The papillae of lanugo hairs were seen to contain dendritic melanocytes whose processes extended directly into the base of the hair shafts. The melanization of hairs occurred independently of the so-called epithelial matrix, which itself became pigmented later on. The "interlacing pigment cells" noticed by Thomson evidently correspond to the dendritic melano-cytes, which lie between ordinary matrix cells of the hair bulb.

These conditions were further studied by Zimmermann (1954), who also attempted the first evaluation of the numerical density of melanocytes in the fetal epidermis of Negroes. The intercellular distances of 200 consecutively encountered melanocytes were measured in serial sections. These distances decreased by approximately one-half between the fourth and the fifth months. It was concluded that the number of dendritic cells had doubled during that interval.

Becker and Zimmermann (1955) carried that numerical analysis further. Cell counts were made in spreads of separated epidermis. In the newborn Negro they found approximately 1,000 dopa-positive melanocytes per mm². This figure compares favorably with similar counts made by Szabo (1954) in adult white skin. Gold chloride impregnations revealed the earliest dendritic melanocytes in the epidermis of white fetuses at six months. In Negro fetuses of the third month the first mature melanin granules were identified in melanocytes of the eyelids, the external auditory meatus, and specific areas of the oral mucosa.

Hu, Staricco, Pinkus, and Fosnaugh (1957) made observations on melanocytes of the prepuce of white and Negro infants. Their illustrations of cells in tissue cultures show many types resembling those described in the present study. They found that "the relatively small pigment cells in the outgrowth of normal skin explant resemble the early melanoblasts of fetal life reported by Zimmermann and Cornbleet." Young pigment cells were recognized in the cultures as bipolar or stellate cells. As they matured they became strongly dopa positive and showed richer dendritic ramifications. There were no transitional forms between ordinary epithelial cells and melanocytes. Each cell type gave rise to daughter cells of its own kind.

(d) *Evidence of gradients in the development of cutaneous pigmentation*. Although the migration of melanoblasts from the neural tube to various body regions had been well established for certain vertebrates, there is only fragmentary information on the rate at which they arrive at ultimate destinations. Hopkins-Fox (1949) first determined such a schedule in embryos of barred Plymouth Rock chicks. The migratory melanoblasts could not be identified with certainty in histological preparations. The evidence, therefore, was based on the end results of grafting experiments. Of more than 2,000 transplants, about 1,000 were successful. The earliest migration of melanoblasts from the neural tube occurred at the level of the mesencephalon, in chick embryos of 8 to 10 somites. In embryos with more than 27 somites "the epidermis overlying all somites tended to yield melanoblasts upon transplantation." Roughly, an antero-posterior sequence in the migration of melanoblasts was revealed. In the limb-buds there was a proximo-distal gradient as well as a dorso-ventral migration. The migration was not limited to the epidermis, but melanoblasts also reached visceral structures along blood vessels (coelomic lining, mesorectum, testes).

(e) *Experimental evidence for the origin of mammalian melanocytes from the neural crest*. The fundamental work by Rawles (1947, 1948) was briefly referred to in the introduction. The pigment-forming potency of various body regions of mouse embryos of a black strain was tested by transplanting them into the coelom of white Leghorn (albino) chick embryos. Only tissue grafts that contained presumptive or definitely identified neural crest were able to differentiate melanocytes. A medio-lateral spread in that pigment-forming capacity occurred, first at cranial and later at caudal levels. By several hundred grafting experiments Rawles proved conclusively that the somatic ectoderm or its hair follicles are incapable of producing their own melanin. In mammals, too, that faculty belongs exclusively to melanoblasts and melanocytes, which are derived from the neural crest.

Such experimental proof is not feasible in man. A search for the earliest phases of melanogenesis by histological means, therefore, appears to be the only possible approach. The results of our own endeavors are presented in the following pages.

III. Materials and Methods

One hundred and seven Negro fetuses were collected through the courteous co-operation of various hospitals and institutions: the Department of Obstetrics and Gynecology of the Illinois Research and Educational Hospitals (Dr. W. F. Mengert), the Department of Pathology of

the University of Illinois, College of Medicine (Dr. C. A. Krakower); Cook County Hospital, Department of Pathology, Chicago (Dr. P. B. Szanto); the Carnegie Institution of Washington, Department of Embryology, Baltimore (Dr. G. Corner); Tulane University, New Orleans, Department of Anatomy (Dr. H. Cummins) and the Department of Medicine (Dr. V. Derbes); Los Angeles County General Hospital, Department of Surgical Pathology (Dr. W. Bullock); the Chicago Maternity Center (Dr. Beatrice Tucker), and the Moline City Hospital, Illinois (Dr. N. T. Braatelien).

We extend our sincere thanks to all those who made this material available to us.

About 60 specimens were particularly well preserved. All were formalin-fixed. Detailed records were kept concerning the findings on melanocytes in the dermis and epidermis. Other specimens were discarded because they were too young, showed various degrees of maceration, or were otherwise inadequate for our study.

Age determinations were made from careful measurements of crown-rump length and by applying the formulae of Scammon and Calkins (1929):

$$C.R. \text{ Length} = .66 \text{ C.H. Length (cm)} + .5 \text{ cm}$$

and

$$Age = \left[\frac{C.H. \text{ cm}}{28} + 1.25\right]^2 + .74$$

These rules give menstrual age in lunar months of 28 days.

The ages of several young fetuses obtained from the Carnegie Institution of Washington had been established according to the rigorous criteria of that laboratory. Our own calculations coincided with theirs. We were able to obtain only skin specimens from several older fetuses (twenty-sixth to twenty-eighth week) and newborn Negroes from Cook County Hospital, Chicago. We accepted the ages given by the hospital's Department of Pathology.

Wherever feasible we made skin shavings from 21 selected body regions of each fetus: two from scalp and cheek, seven areas from the trunk, six dorsal and volar areas of the upper limb including the palm, and six posterior and anterior regions of the lower limb, including the sole. In fetuses of the third and fourth months, the full thickness of the delicate skin could be used. In older fetuses, two horizontal slices usually were made, one containing the epidermis with the upper portion of the dermis and another one consisting of the deeper dermal layer. By stretching the skin areas of the specimens it was possible to make the slices by hand, merely using a razor blade. After staining, the preparations were mounted as spreads, alternately with the dermal or the epidermal surfaces upper-

most. Surface examinations of such spreads clearly revealed the number and manner of arrangement of melanocytes. Population densities per mm² could be determined more accurately than is possible in sectioned material.

Our extensive slide collection was prepared by Dr. Hans J. Knoblich. We are glad to acknowledge our indebtedness for his fine technical assistance and faithful co-operation.

Staining Technique. The best results were gained by modifying Masson's impregnation method of ammoniacal (reduced) silver nitrate. After thoroughly washing the formalin-fixed skin slices in distilled water, we placed them in a freshly prepared and filtered solution of 10 per cent ammoniated silver nitrate. Masson's original procedure required 6–8 hours impregnation time at room temperature. We incubated our preparations at 55° C. from 10 to 30 minutes. Frequent checking of the specimens (in distilled water, under the microscope) prevented overstaining. Usually a sepia-brown tint of the skin slices indicated that the incubation could be terminated. Even in such non-cleared preparations the epidermal melanocytes could be readily detected by low power examinations. The quality of the fixation appeared to affect the staining time.

Adequately impregnated skin spreads, usually about 1 cm² in size, were then treated with a 6 per cent solution of sodium hyposulfite. Gold toning was omitted. The preparations were then dehydrated in an alcohol series, placed in xylol, and mounted in "Permount" as spreads.

The study of vertical skin sections was accessory. Sections were used mainly for the accurate determination of the depth at which melanoblasts and incompletely differentiated melanocytes were found in the dermis. These sections also were impregnated with ammoniated silver nitrate and incubated at 55° C. The stain was no better, but much faster than that produced by the original Masson technique. After gold shading, the preparations were finished routinely.

Cell Counts. Cell counts were made in over 500 microscopic fields of epidermal and dermal melanocytes. All counts were obtained by means of camera lucida projections. The standard field measured $(0.33 \text{ mm})^2 = 1/9 \text{ mm}^2$. Each observed melanocyte was traced, and after the field was completely surveyed the sketched-in cells were counted. Their number was then multiplied by nine to obtain an estimate of their population density per mm². Obviously, any error in counting was also multiplied by nine. However, checks were made by tracing and counting the cells of one particular field on ten different plots. At other times the cell counts in a given field were made by different observers. In either case the errors were negligible, primarily because the silver impregnations were of high quality and the melanocytes easily identified. A comparison of cell counts in any two fields appeared justified and reliable.

The senior author is responsible for practically all cell counts and for the analysis of the data.

The photomicrographs were made by Mr. Lawrence Toriello, Illustration Studios, University of Illinois. His skillful work is greatly appreciated.

IV. Melanoblasts and Melanocytes in the Fetal Dermis

We searched for precursor stages of melanocytes in many body regions of 54 selected specimens. Many of these fetuses were also used for the study of epidermal melanocytes reported on in succeeding sections.

Questions of nomenclature were discussed in the introduction. We are using the term "melanoblast" for an embryonic type of cell, potentially able to produce melanin. A "dermal" melanocyte is a more highly differentiated cell, fusiform or stellate in shape, containing premelanin or melanin, and is located in the dermis. It is also designated as an immature melanocyte. An "epidermal melanocyte" is the fully differentiated, dendritic type of cell, also elaborating melanin and located exclusively in the epidermis.

"Dermal" melanocytes become visible in *unstained* fetal skin spreads as early as the fourth month (figure 7). Their granules are undistinguishable from melanin and have affinity for reduced silver nitrate (Masson).

Dopa reactions were not feasible in our formalin-fixed material.

Tenth and Eleventh Weeks of Fetal Development. The earliest melanoblasts were identified in skin specimens of ten Negro fetuses of this developmental period. Their crown-rump length ranged from 3.4 to 4.5 cm. Preparations from various body regions were obtained by stripping small pieces of the delicate skin. In eight fetuses of this group the melanoblasts were found only in the scalp, in others also in the nape and in the sacral region. They were identified as round cells of from 8 to 12 μ diameter. Their eccentric nucleus usually contained one or two nucleoli. Fine argentaffin granules were disseminated throughout the cytoplasm. Due to the spherical shape of these cells, they appeared to be more densely arranged at the periphery.

The relatively large melanoblasts were widely dispersed in the connective tissue. At that stage of development a true dermis cannot be distinguished from the hypodermis. Cell counts were not made because of the scarcity and wide scatter of these elements. The earliest precursors of future pigment cells were also recognized in stripped skin pieces simply mounted in water and examined under the microscope. Such cells contain refractile granules which make them readily identifiable. The size of the refractile granules corresponded to that of the argentaffin granules seen after impregnation with reduced silver nitrate.

Changes in form of the round melanoblasts occur already during the tenth week of development. Some were ovoid, assuming the shape of falling drops or of lemons with two small projections at each pole. Gradually more and more fusiform cell types appeared, often of 20 μ length. The argentaffin granules tended to accumulate in the tips of the cell processes, giving the impression of active "growth points." Some ovoid cell bodies had two processes at one pole, foreshadowing a tripodal arrangement of future dendritic processes.

Common to all forms were the argentaffin granules of very fine, even size. Their presence and the continuous series of cell shapes from round to stellate forms were the cytomorphogenic features indicating a single lineage. In scalp spreads of the eleventh week some spindle-shaped cells measured between 30 and 45 μ as compared with an average diameter of only 15 μ of the overlying epidermal cells. In general, the differentiating melanocytes of the dermis were conspicuously larger than fibroblasts or fibrocytes of their surroundings.

During the tenth and eleventh weeks of development, melanocytes in the epidermis were rare. Round melanoblasts and immature melanocytes in the fetal dermis, therefore, precede the first appearance of epidermal melanocytes (in numbers) by about two weeks.

Twelfth Week. In nine fetuses of this period, melanoblasts and immature melanocytes were identified in many body regions. We consider this as an indication of the rapid migration or arrival of pigmentary precursor cells from their presumptive source in the neural crest. The crown-rump length of the specimens varied from 5.0 to 6.7 cm. Since the skin was still very delicate, full-thickness strips could be used. Melanoblasts and transitional forms of "dermal" melanocytes were observed in the scalp, cheek, nape, interscapular, and sacral regions, in dorsal areas of the forearm, of hand and foot, anterior aspect of the leg, and even in the palm and sole.

The first cell counts of dermal melanoblasts and of incompletely differentiated melanocytes were feasible. The distribution of "dermal" melanocytes, however, was not uniform through a given field. They appeared in groups or "streams" of considerable accumulations. Often the long axes of the spindle-shaped cells were parallel to each other, indicating, perhaps, a directional flow through the connective tissue spaces.

The cell counts given in Table 1 cannot be taken as an absolute measure of population densities. They merely indicate the relative frequencies with which melanoblasts and "dermal" melanocytes were encountered at this early age. The table contains the calculated number per mm^2 of both "dermal" and epidermal melanocytes. The great variability in the number of epidermal pigment-producing cells is due to their irregular distribution pattern at this early age.

TABLE 1. CELL COUNTS OF MELANOBLASTS AND EARLY
EPIDERMAL MELANOCYTES IN THE TWELFTH WEEK

Body regions	Melanoblasts and "dermal" melanocytes per mm²	Epidermal melanocytes per mm² in corresponding areas
Scalp	63, 182, 198, 387	72, 189
Interscapular	198	90, 141, 180, 315
Forearm, dorsum	45, 162	171
Hand, dorsum	144, 252	90, 135
Palm	198	234, 279, 351
Leg, ant. region	126	90, 36
Foot, dorsum	378	18
Sole	162	63, 72, 153, 243

The wide range of cell counts in a given region is due partly to the migration and arrival of melanocytes in irregularly scattered groups or "swarms." Stabilized and more typical distribution patterns of melanocytes become established in the fourth and fifth fetal months.

Numerous micrometric measurements were made with high power magnification. The spherical melanoblasts measured from 8 to 10 μ, teardrop or lemon-shaped forms from 12 to 16 μ. Cells with stubby processes varied between 20 and 25 μ. Spindle-shaped forms of immature melanocytes measured from 30 to 45 μ in length.

Figures 3 to 6 show high power photomicrographs of a round melanoblast and of early forms of "dermal" melanocytes (twelfth week). Obviously, sharp focussing of these cells in the dermis of skin spreads is more difficult than in sections.

Thirteenth and Fourteenth Weeks. The six fetuses of this fetal period varied in crown-rump length from 7.0 to 9.5 cm. Melanoblasts and "dermal" melanocytes now were present also in the pectoral region and on the anterior aspects of arm and thigh. Figure 9 illustrates early forms of melanocytes in the dermis of a lumbosacral spread.

Cell counts varied generally between 200 and 400 per mm². In one fetus, however, the counts were much higher in the sacral region and in the dorsum of hand and foot. Specifically, the counts were 684 and 1,395 for the sacral region, 693 and 1,161 for the dorsum of the hand, and 855 in the dorsum of the foot. Counts of the same cell types in the palm and sole were the lowest for that specimen: 198 and 261, respectively.

During the thirteenth week young epidermal melanocytes appear in many skin regions. Such juvenile forms usually are slender, fusiform cells with long primary dendritic processes. They are easily recognized in a higher focal plane than that of the "dermal" melanocytes. The latter also

tend to be spindle-shaped with cell processes just beginning to form. Fusi-form "dermal" melanocytes from the dorsum of the foot are shown in figure 8.

Fifteenth and Sixteenth Weeks. The seven fetuses of this developmental period, terminating the fourth month of pregnancy, varied in crown-rump length between 9.5 and 11.7 cm. The population density of "dermal" melanocytes, in general, was higher than in the preceding weeks, although there still was considerable variation. This may have been due partly to technical procedures. If surface shavings are too thin the preparations may reveal only part of the population of dermal melanocytes. Cutaneous areas from the scalp, and dorsum of the hand and foot, sometimes contained 500 to 700 melanoblasts and "dermal" melanocytes per mm^2. Spindle-shaped forms usually were predominant, although in one fetus of 16 weeks many round melanoblasts were typical of various regions, particularly in the dorsum of hand and foot.

During this developmental period, "dermal" as well as epidermal melanocytes become visible in *unstained* preparations. Figure 7 shows spindle-shaped cells in the dermis of an unstained scalp spread (fifteenth week.) The presence of true melanin in those cells was proved by impregnations with ordinary silver nitrate (Bizzozzero's method). Relatively young forms of "dermal" melanocytes from the dorsum of the hand are shown in figure 10.

Seventeenth to Twentieth Weeks. Split-skin preparations were made of 15 fetuses of the fifth month of pregnancy. Their crown-rump length varied between 12.5 and 16.5 cm.

Cell counts of "dermal" melanocytes remained high in most regions. There were over 1000/mm^2 of such cells in the sacral region (Mongolian spot) of two specimens (figure 11). Counts of epidermal and "dermal" melanocytes of a preparation usually were lower by one-half or two-thirds for the latter type of cells, except in the sacral region. Cell counts in preparations impregnated with reduced silver were consistently higher than those in unstained skin spreads. This agrees with the generally accepted view that ammoniacal silver nitrate reveals precursor stages as well as fully elaborated melanin granules. In unstained preparations presumably only the latter are visible.

In some fetuses of this period, the spindle-shaped melanocytes in the dermis were the most characteristic cell type. They had become longer and measured from 40 to 80 μ. In other specimens the majority of the "dermal" melanocytes were of the round or ovoid form. They may have been "young arrivals" in the particular regions (lumbosacral, dorsum hand, dorsum foot).

During this developmental period, immature melanocytes also tended

to become adherent to small blood vessels. Whole chains clearly outlined
the course of capillaries. The cells often had the appearance of silver-
impregnated "pericytes." Figure 12 portrays such an arrangement in the
dermis of the scalp. Migratory melanocytes may also follow the course of
nerves in the dermis. Such migration, however, seems to be incidental
rather than essential. Similar observations by Ehrmann may have induced
him to believe that melanoblasts were derived from the adventitia of blood
vessels.

Sixth Month to Birth. The material for this period consisted of three
specimens of the twenty-sixth and twenty-eighth weeks and of four full-
term fetuses. In the full-term fetuses the epidermis was sloughed; the
dermis, however, was well preserved and excellent silver impregnations of
"dermal" melanocytes were obtained.

Characteristic of this late fetal period is the disappearance of the
melanoblasts and melanocytes from the dermis of most skin areas. Per-
haps the cells merely lose the active enzyme system that is necessary for
the elaboration of melanin. They would then become undetectable with
our present methods. They may disappear entirely, though we cannot
be sure.

Equally characteristic, however, is the fact that "dermal" melanocytes
remain a constant feature of the scalp, the sacral region, and the dorsum
of the hand and foot. In the last two regions their number varied between
500 and 600 cells at six months as well as at birth. In the sacral region of
several newborn specimens we counted from 900 to 1,400 "dermal" mela-
nocytes per mm.^{2} Figure 13 shows such cells from the dorsum of the hand
of a newborn Negro.

V. The Development of Distribution Patterns of Epidermal Melanocytes During the Fetal Period

It has been known for some years (Zimmermann and Cornbleet, 1948)
that the first epidermal dendritic cells appear early in the third fetal
month. Becker and Zimmermann (1955) had also established that in the
newborn Negro there are approximately 1,000 melanocytes per mm^2 of
epidermis. But only incomplete information has been available concerning
the population density of such cells at different fetal periods and in various
skin areas.

Previous data were based on skin sections in which the mean distance
between 200 consecutively encountered melanocytes had been measured.
The probable number of melanocytes per mm^2 was then estimated. It is
difficult to identify functionally immature dendritic cells in skin sections
of early fetal months. In trying to avoid that the same cells were counted

in adjacent sections, we had measured only alternate sections. This pre-caution led to cell counts that were too low. Our more reliable, present method calls for correction of the figures published in our 1955 study.

Forty-three fetuses of our material had been aborted between the tenth and twentieth weeks of development. This developmental period proved to be an important one for the differentiation of epidermal mela-nocytes and the establishment of their regular distribution pattern. No intraepidermal dendritic cells could be seen before the tenth week (fetuses with a C.R.L. of 40 mm or less). In the twentieth week, however (C.R.L. of over 160 mm) the population density of epidermal melanocytes in most skin areas already resembled the conditions at birth (800–1,100 per mm²). The following is an account of the development of distribution patterns of dendritic cells until birth.

Tenth Week. The earliest epidermal melanocytes were identified in the interscapular region of a fetus measuring 34 mm in crown-rump length. Its age was calculated at 2.7 lunar months or 10 3/7 weeks. Widely scat-tered dendritic cells were observed, some with fairly long, branched proc-esses. Figure 14 shows one of these earliest intraepidermal cells. Three dendritic processes extend through a total distance equal to about ten ordinary epidermal cells. Argentaffin granules of premelanin became vis-ible with Masson's reduced silver technique. They could not be seen in unstained preparations nor after treatment with ordinary silver nitrate (Bizzozzero). Fully elaborated melanin, therefore, was not present. The dermis contained numerous round melanoblasts, e.g., in the sacral region. Counts of the sporadically appearing, earliest melanocytes of this stage would be meaningless.

Eleventh Week. Scattered intraepidermal melanocytes were observed in various cutaneous areas of eight fetuses of this period. Their crown-rump length varied between 37 mm and 55 mm. Scalp, cheek, and nape revealed dendritic cells in most of these specimens, although never in high numbers. A few cells were also identified in the interscapular, para-umbilical, and gluteal regions. Even the epidermis of the delicate limbs, on the dorsum of the arm and the posterior region of the thigh, contained a few young dendritic cells. In most areas there were fewer than ten early melanocytes per mm². Cell counts were not reliable. The youngest forms were fusiform with slender, drawn-out cell bodies and two dendritic proc-esses. Some were triangular, with indications of three processes, or even with secondary branches. Some of the processes measured 50 μ in length. The total length of the fusiform cells usually ranged between 50 and 75 μ. The distal limb segments were still devoid of dendritic cells.

Twelfth Week. Epidermal melanocytes now appeared also in palm and sole. The distribution of early dendritic cells was regular enough to

allow cell counts in five of the seven available fetuses of this period. Their crown-rump lengths varied between 50 and 67 mm. The cell counts ranged from 30 to 200. In two specimens, however, the population density of young dendritic cells already was remarkably high, e.g., 576 cells per mm^2 in the nape and 738 cells per mm^2 in the sole of one, 603 cells in scalp and 513 in the palm of the other specimen. Since the distribution pattern was uneven, these counts convey at least an impression of the rapid migration of melanocytes into specific epidermal areas. Transitional cell forms between young dendritic cells and ordinary epithelial cells were not seen. Each of the 20 skin areas contained some dendritic cells at this early age. Their cytodifferentiation occurred rapidly. Figures 15 and 16 represent early cell forms and distribution patterns of epidermal melanocytes of a twelfth week fetus. The fusiform cell bodies of the sole, showing long primary dendritic processes, were characteristic of young dendrities in all cutaneous areas. In the sole they had just appeared at this age; in the nape (figure 16) they had already attained a higher degree of differentiation.

Figures 17 and 18 represent cell stages encountered in full-thickness skin spreads of two fetuses obtained from the Carnegie Institution of Washington, Department of Embryology (Baltimore). Their respective ages had been determined in that laboratory as 12 weeks (Carnegie specimen No. 9014) and 12 3/7 weeks (Carnegie specimen No. 8613). Figure 17 shows highly differentiated melanocytes in the epidermis of the scalp and figure 18 of the anterior region of the leg. The surface views of these spreads show the nuclei of ordinary epidermal cells and permit a comparison with the length of the dendritic processes. The dendritic cells reveal accumulations of argentaffin granules to the tips of their processes.

Figures 19 and 20 portray conditions of melanocyte differentiation in the nape and in the dorsum of the arm. The cells are of a uniform type in the former but variable in the latter region. The fusiform, slender cell forms probably had recently "arrived" in the epidermis on the dorsum of the arm.

A relative "timetable of arrival" of epidermal melanocytes in different skin areas of an individual is shown by cell counts. During the twelfth week they were consistently higher in the palm than in the sole, e.g., 351:72; 234:63 per mm^2.

Thirteenth and Fourteenth Weeks. A marked increase in the number of epidermal melanocytes characterized this period. In the palm appeared the first indications of rete ridges (epidermal crests).

We studied six fetuses ranging from 66 to 75 mm C.R.L. in the thirteenth week and from 85 to 133 mm in the fourteenth week.

The distribution of young dendritic cells was more uniform and cell

counts became reliable. Cell distribution remained irregular in only a few skin areas. The orientation of melanocytes often indicated a directional flow or migration (figure 20). Population densities of the dendritic cells varied, both regionally and individually, between 300 and 1,000 per mm². In the scalp, cheek, dorsal trunk regions and dorsum of forearm the counts were between 800 and 1,000 cells. Great waves of rapidly differentiating dendritic cells thus appear to arrive at their destination during a short period of only two or three weeks. Their population density quickly approached that of the newborn. Since nuclear events in epidermal melanocytes cannot be observed after silver impregnations, we are unable to say whether the increase in cells was due, in part, to mitotic divisions.

Figure 21 illustrates the distribution pattern in the scalp of a Negro fetus of 13½ weeks (75 mm C.R.L.). We counted 936 dendritic cells per mm² of that area.

The distribution features in palm and sole were of special interest. When they first appear the melanocytes are fairly evenly scattered throughout the epidermis of palm and sole. Figure 23 shows that pattern in the palm at 13 weeks. It changed rapidly with the differentation of rete ridges (epidermal cristae) between the thirteenth and seventeenth weeks. This occurs first in the palm. Figure 24 shows the dendritic cells located almost exclusively on the rete ridges. Palm and sole contain some melanocytes until birth and even to adult life. Their potentiality to form melanin, however, appears more inhibited than elsewhere in the body.

Fifteenth and Sixteenth Weeks (end of fourth month). Our material consisted of three fetuses of the fifteenth week (95 to 105 mm C.R.L.) and of four fetuses of the sixteenth week (107 to 117 mm C.R.L.). In general, the distribution patterns of the dendritic cells were regular and uniform in all skin areas, except in the palm. Cell counts were higher than in preceding periods. This reflects a continued infiltration of the epidermis by melanocytes from the dermis. At 15 weeks the population density of dendritic cells varied between 600 and 1,200 per mm². Figure 22 shows a characteristic distribution pattern of epidermal melanocytes in the interscapular region at that time.

In *unstained* spreads of scalp specimens of the fifteenth and sixteenth weeks, the dendritic melanocytes were faintly visible. The degree of their differentiation corresponded to that shown by impregnations with ammoniacal silver nitrate. Unstained dendritic cells were also identified at the palpebral fusion plate. Impregnations by Bizzozzero's method proved that they contained fully elaborated melanin. We had previously shown that melanin is first formed in melanocytes of certain head regions early in the fourth month.

The rete ridges in the palmar epidermis become well established during

the fifteenth week. The previously scattered melanocytes now were located on the ridges and their number decreased to 150–200 cells per mm². This may be due to a loss of stainability or to actual disappearance of the dendritic cells. During the fifteenth week the sole usually has no rete ridges as yet.

During the sixteenth week, cell counts of epidermal melanocytes varied between 700 and 1,200 per mm². The palmar and plantar areas were again the exceptions. In the palm all the dendritic cells were on the rete ridges and numbered between 150 and 250 cells per mm². In the sole, instead, the epidermal cristae were just beginning to form and the number of the evenly scattered melanocytes remained relatively high: 375 to 450 per mm².

Seventeenth and Eighteenth Weeks. There were no important changes in this developmental period. Specimens of the seventeenth week measured between 125 and 129 mm in C.R.L. and between 130 and 137 mm C.R.L. in the eighteenth week.

In general, the population density of melanocytes was similar to that at the end of the fourth month of development (600–1,200 cells per mm²). There were two exceptions. Counts in skin spreads of a 130 mm fetus were consistently low (300–700 cells). There were some signs of relatively poor preservation and the counts may not be reliable. Unusually high cell counts were obtained in preparations from a fetus of 17 weeks; many exceeded 1,000 per mm². Fixation and silver impregnation were good. We ascribe such high counts of dendritic cells to individual variation, which will be discussed in a subsequent section.

During the seventeenth and eighteenth weeks, the development of rete ridges in palm and sole had further progressed and constituted very characteristic features. The melanocytes were located exclusively on the ridges. Their number was consistently higher in the sole than in the palm. Cell counts in both areas were higher in specimens of the seventeenth than in the eighteenth week. In the palm the average number per mm² decreased from about 200 to 100, in the sole from about 400 to 175 cells. This may be due to a loss in functional activity with resulting unstainability, or it may mean an actual decrease in the number of melanocytes.

Figure 24 shows the arrangement of the melanocytes on the epidermal ridges in the palm of a Negro fetus of 17 weeks. There were 126 dendritic cells per mm² (compare with figures 23 and 26).

Nineteenth and Twentieth Weeks (end of fifth month). Split-skin preparations were obtained from five fetuses belonging to the nineteenth week of development (145 to 150 mm C.R.L.) and of two fetuses of the twentieth week (165 mm C.R.L.). Cell counts from one fetus of the twenty-first week were included in this period.

The population densities of epidermal melanocytes varied between 500 and 1,000 cells per mm². In one fetus it ranged from 700 to 1,100 and in another between 800 and 1,400.

The impression gained from an analysis of cell counts in the seventeenth and eighteenth weeks was sustained: the great influx of melanocytes into the epidermis had occurred before the end of the fourth month. There was now a distinct slowing of that process. A degree of stabilization appeared to be attained, although various cutaneous areas still contained melanocytes in the dermis. Barry (1953) had noticed it but nothing is known of the causes. We can merely state that the period of penetration of the epidermis by melanocytes is relatively short and occurs essentially before the midpoint of pregnancy.

Figure 25 shows a typical distribution pattern of epidermal melanocytes in the anterior abdominal wall at 19 weeks. The population density was 873 cells per mm².

The gradual decrease in the number of melanocytes on the epidermal cristae of the sole is shown by the following figures: 531 per mm² at 17 weeks, 360 at 19, and 270 at 20 weeks.

Twenty-Sixth Week. We were able to obtain skin specimens of various body regions from two fetuses of 6.6 months. The counts of epidermal melanocytes from 10 cutaneous areas of one fetus (220 mm C.R.L.) varied between 700 and 1,100 per mm². In the sole there were only 81 dendritic cells per mm², all located on the rete ridges. In the other fetus of this period (225 mm C.R.L.), the cell counts from seven cutaneous areas ranged between 1,000 and 1,485.

These counts are in good accord with our findings from the end of the fifth fetal month and with those in the newborn. They indicate that no additional wave of melanocyte "arrivals" occurred during the second half of the fetal period. Scattered melanocytes from the dermis may nevertheless enter the epidermis in small numbers.

Full-Term and Negro Infants of the Neonatal Period. The epidermis had been sloughed off in skin specimens of four full-term fetuses. In the palm of one specimen we were able to count 288 melanocytes per mm². Figure 26 shows their arrangement on epidermal ridges, between openings of the sweat pores. In the sole of another specimen there were 171 dendritic cells per mm². Although unsatisfactory for an over-all study of pigment cells in the epidermis, the four fetuses nevertheless revealed great numbers of melanocytes in the dermis of special areas. Reference to those findings was made in another section.

Reliable counts of melanocytes in the newborn had been previously reported from the abdominal wall (Becker and Zimmermann, 1955). Epidermal spreads had been obtained by trypsin digestion. They were fixed

in 2 per cent formalin for four hours and then incubated in a 1 per cent dopa solution for three hours. Counts ranged between 983 and 1,152 dopa-positive dendritic cells per mm². In a ten-day-old Negro infant, the dopa-treated epidermis contained between 617 and 814 melanocytes per mm² of abdominal surface area.

VI. Regional Differences in the Frequency Distribution of Epidermal Melanocytes in the Negro Fetus

A total of 412 field counts of epidermal melanocytes were made. Only such preparations were used in which the melanocytes were well impregnated with reduced silver nitrate. There were differences in the intensity with which individual cells took up the silver. Since all preparations were made with equal care, our numerical data are considered adequate for an estimate of regional differences in population densities. They represent a first attempt at determining the time of arrival of fetal melanocytes in various body regions. Wherever feasible 20 cutaneous areas of each fetus were studied.

Figure 1 graphically illustrates the pooled data for two developmental periods: (a) the twelfth week, when the epidermis is being invaded by melanocytes in numbers, and (b) a longer interval from the thirteenth to the twenty-first weeks (end of fifth fetal month). During that time occurs a stabilization of the cell counts. Consequently, the population density of epidermal melanocytes remained relatively constant during the second half of pregnancy.

During the twelfth week, cell counts in different regions varied from about 20 to 400 dendritic cells per mm² (see Table 1). A total of 50 counts were made. The highest counts were obtained from the scalp, cheek, nape, and interscapular regions. The counts for various cutaneous areas of the upper limb were higher than those of the lower limb. Surprisingly high numbers of dendritic cells were obtained for palm (351/mm²) and sole (243/mm²).

Since the head region of an embryo and young fetus grows earlier and faster, and the anterior limb differentiates ahead of the posterior limb, the population densities of melanocytes appear to conform with the general sequence in cephalo-caudal growth. An antero-posterior gradient in the distribution density of early melanocytes was clearly indicated.

Relatively high counts in the palm and sole may be due to an accumulation of migratory melanoblasts in distalmost areas. Their invasion of the epidermis and differentiation into dendritic cells may occur at a high rate. The population density in the palm and sole increased during the thirteenth week but decreased thereafter. A dorso-ventral difference in the number of melanocytes was not evident in the twelfth week.

COMBINED AVERAGE COUNTS OF DENDRITIC MELANOCYTES PER MM²
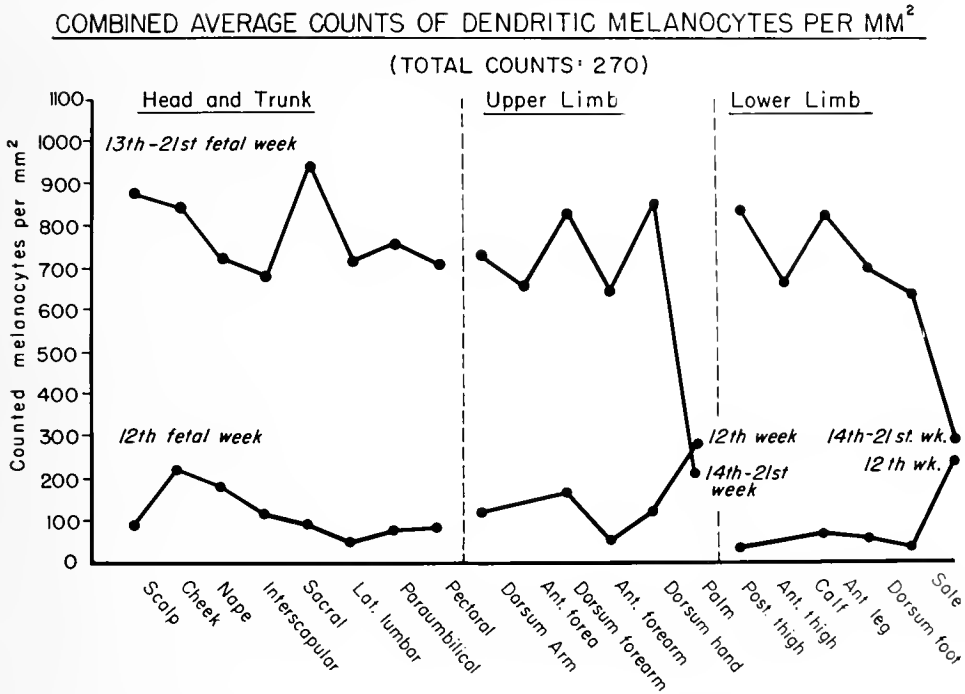
(TOTAL COUNTS· 270)

Figure 1. Population densities of epidermal melanocytes in various body regions. The lower curve shows average counts of melanocytes per mm² during the twelfth fetal week. The upper curve represents pooled frequencies between the thirteenth and twenty-first fetal weeks. The number of melanocytes in palm and sole drops sharply after the thirteenth week.

The top portion of the graphs in figure 1 shows the average frequency distribution of epidermal melanocytes in 20 areas of fetuses between the thirteenth and twenty-first week. It is based on 270 field counts of dendritic cells.

The data were first plotted separately for the thirteenth week (25 counts), for the fourteenth to sixteenth week (149 counts) and for the nineteenth to twenty-first week (96 counts). Since there was an overlap of population densities in corresponding areas we pooled the entire group of data. The graph portrays the main trend of regional cell frequencies during the fourth and fifth fetal months.

In fetuses of the thirteenth week the counts of epidermal melanocytes were significantly lower for the lower limb as compared with those of the upper limb. This agrees with the slower or delayed rate of development of the hind limb. In the following few weeks that difference in population densities became rapidly erased.

Between the thirteenth and twenty-first weeks the average counts of

melanocytes for all cutaneous regions varied between 650 and 950 mm². The greatest influx of epidermal melanocytes into any of these areas had occurred between the twelfth and fourteenth weeks of intrauterine development.

The most characteristic features were the gradual disappearance of a cephalo-caudal gradient, and the clear emergence of a dorso-ventral gradient in population densities, for the trunk, upper and lower limb.

The counts of melanocytes did not remain significantly higher in the cephalic portions of the fetus. High counts were obtained from the sacral region. The number of dendritic cells in dorsal areas of both limbs was also approaching the previously higher counts in the head region.

The pooled average counts from the dorsal areas of trunk and limbs differed from those of corresponding ventral areas. They were significantly higher in the sacral area, dorsum of arm, forearm and hand, posterior region of thigh, and in the calf, than those of the paraumbilical and pectoral regions, or of anterior areas of arm, forearm, thigh, and leg. This is clear evidence of a dorso-ventral gradient in the population densities of melanocytes at that fetal period.

The high counts in the sacral region (average: 947/mm²) are of special interest since that area corresponds to the caudal end of the neural tube and of the neural crests. Great numbers of pigmentary precursor cells accumulate in the dermis of that area. They constitute the substrate for the so-called Mongolian spot. Many of these cells may later penetrate the epidermis and thus lead to a high population density of dendritic cells in the sacral area. All the epidermal melanocytes of the lower limb, incidentally, must also be derived from the dermal pool of melanoblasts in that area.

Special features prevailed in the epidermis of palm and sole of the fourth and fifth months. The first epidermal cristae or rete ridges appear in the palm about two weeks earlier than in the sole. They were easily identified in both areas during the eighteenth week. None were present elsewhere in the skin of that period. During the formation of rete ridges, the counts of palmar and plantar melanocytes dropped sharply. Their distribution pattern changed from one of evenly scattered cells to one of restricted alignments on the rete ridges. There remained only rare dendritic cells between the epidermal crests. This decrease is perhaps more simulated than real; if the enzymatic activity necessary for melanin production became inhibited, the cells would not be revealed by our impregnation technique. Inhibition of tyrosinase activity by SH groups has been proved to occur in adult white skin. In the palm and sole of Negroes it begins perhaps as early as the fourth fetal month.

VII. Individual Variability in the Population Densities
of Melanocytes During the Fetal Period

The pooling of cell counts through a relatively extensive developmental period, as done in the foregoing section, has the advantage of diminishing the effects of minor technical errors and of bringing out the major trends of conditions under study. Such a procedure has the disadvantage, however, of erasing individual differences in the population density of melanocytes. The "average truth" is not the whole truth in these matters, any more than in many others.

A possible source of error resides also in relationship between the degree of differentiation of a specimen and its age as determined by external body dimensions. Streeter showed in his "horizons of human development" that an identical degree of interior differentiation may be attained in embryos of different external dimensions. During fetal development that discrepancy probably is less significant. Our age determinations according to Scammon and Calkin's formulae are valid for comparisons, but they do not necessarily reflect absolute age. Any errors in this respect probably would affect age determinations by not more than one week.

Already in the twelfth week we encountered one specimen in which the average count of melanocytes per mm^2 was much higher (540) than the entire range for other individuals of that period (50–200). A similar difference between specimens of practically identical crown-rump lengths was noted at 15 weeks. The discrepancy in cell counts was especially apparent in corresponding skin areas of the lower limbs. Again in the eighteenth week we obtained range variations in cell counts from 300–700 in one individual and from 600–1,000 in another. The range of variability generally was between 500–1,000 melanocytes/mm^2 in specimens of the nineteenth week. In one individual of the twentieth week the counts varied from 700 to 1,100 cells and in another fetus of the twenty-first week from 500 to 900. In two fetuses of the twenty-sixth week the cell counts (disregarding palm and sole) were between 675 and 1,100 per mm^2 in one specimen, and between 1,000 and 1,485 in the other. Counts in skin specimens of newborn Negroes averaged 1,035 melanocytes per mm^2. Unfortunately we were not able to obtain material from many cutaneous areas of that age.

The above-reported individual differences might be considered characteristic only for fetal stages. Our data do not extend far into postnatal life and we cannot say whether such differences persist. It is well established, however, that they exist both in white and Negro adults.

VIII. Discussion

In the past, cutaneous pigment cells and their precursor stages have been known by various names. To the proponents of a theory that preceded Bloch's, they were fixed connective tissue cells that had penetrated the epidermis. According to Ehrmann, pigment-producing cells were "melanoblasts," located in the dermis or in the epidermis and presumed to be derived from embryonic mesoderm. But Bloch insisted that they were of ectodermal origin, arising in the basal layer of the epidermis. Biologists generally preferred the terms chromatophores or melanophores, evidently disregarding the fundamental difference between carrying melanin and actively producing it.

Adachi, with other distinguished authors of his time, believed that "epidermal chromatophores" were an illusion. Melanin of the mammalian and human epidermis was not contained in cells at all but in intercellular spaces. Others, particularly French authors, designated the epidermal pigmentary elements as "Langerhans cells." Paul Langerhans (1868) had discovered stellate cell forms in adult white skin by means of gold impregnations. He believed them to be nerve cells and did not associate them with pigment production. Later these elements were considered either as artifacts or identical with the true pigment-producing dendritic cells of the epidermis.

According to Masson (1948), "Langerhans cells" represent effete melanocytes that have lost their ability to produce melanin and are approaching desquamation. Billingham and Medawar (1953) emphasized that only "high level" branched cells in the epidermis are identical with "Langerhans cells" and that the latter did not occur in the basal layer. In adult heavily pigmented skin the high level dendritic cells never are dopa positive. They have either lost or discharged their pigment and are an exhausted type of cell.

In fetal Negro skin no distinction can be made between branched cells in superficial or deep layers of the epidermis. All are functionally active as pigment producers. Consequently, there are no "Langerhans cells" in fetal Negro skin. This confirms the interpretation that in adult skin they represent "spent" melanocytes. Hence we have not used the term.

Masson (1948) designated the pigment-producing elements of the epidermis as "clear cells" or "cellules claires." The term stands for cell bodies (perikarya) that lie mostly in the basal layer and usually show some pigmentary activity. They were even described as lymphocytes penetrating the epidermis. Billingham and Medawar (1953) have drawn attention to that erroneous interpretation.

Bloch (1917) and Becker (1927) called the dopa-positive branched cells *Dendritenzellen* or dendritic cells. These terms were particularly

useful in the earlier days of pigment research when neither their origin nor true nature was known. "Dendritic cell" now is a widely used term (Billingham, 1948). It is descriptive, noncommittal, and applicable to all epidermal stellate cells, whether they have pigmentary activity or not.

In this bewildering array of more or less synonymous terms — which naturally has led to confusion — the best name for pigment cells is "melanocytes." We have applied it both to immature, branched forms of pigment-producing cells in the fetal dermis and to the highly differentiated dendritic cells of the epidermis. Pigmentary as well as inhibited dendritic cells are melanocytes. Melanoblasts, instead, are early embryonic cell types, round or ovoid, and reveal the first signs of their pigment-producing potentiality.

During the past 75 years a broad concept has evolved relative to melanocytes in the human dermis. At first recognized only in the sacral region of Japanese fetuses and children, then in the dermis of certain primates, the same cells were subsequently described in the sacral region of whites, in blue nevi, and finally in the dermis of several body regions of early fetuses of the yellow race. We have shown that during early fetal months, immature melanocytes are present in the dermis of all body regions of Negro fetuses. Measurements of the characteristic cells correspond closely to those given by previous authors for the so-called Mongolian cells. Barry (1953) postulated that "dermal melanocytes are the forerunners of dendritic Langerhans cells." He further emphasized that the pigment cells of the Mongolian spot, of the blue nevi, of the hair matrix, and the dendritic cells of the epidermis are cytogenetically related to each other. In general, we agree with this conclusion.

We have found that in later fetal life and at birth, "dermal" melanocytes remain identifiable only in certain skin areas: in the scalp, the sacral region, and the dorsum of the hand and foot. Temporarily, all cutaneous areas of Negro fetuses contain some pigment-producing cells in the dermis. These melanocytes appear in every way homologous to the permanently present pigment cells in the dermis of certain anthropoid apes and monkeys.

Their gradual disappearance from the dermis of most skin areas needs further investigation. The present methods did not permit us to establish whether many of the melanocytes degenerate or whether their enzyme system merely becomes inactive. Possibly some of the cells ascend to the dermo-epidermal junction and mature into highly branched epidermal melanocytes.

Figure 2 shows our interpretation of the paths followed by melanoblasts and melanocytes. Since melanoblasts were not seen before the tenth week of menstrual age, parts A and B of the diagram remain hypothetical.
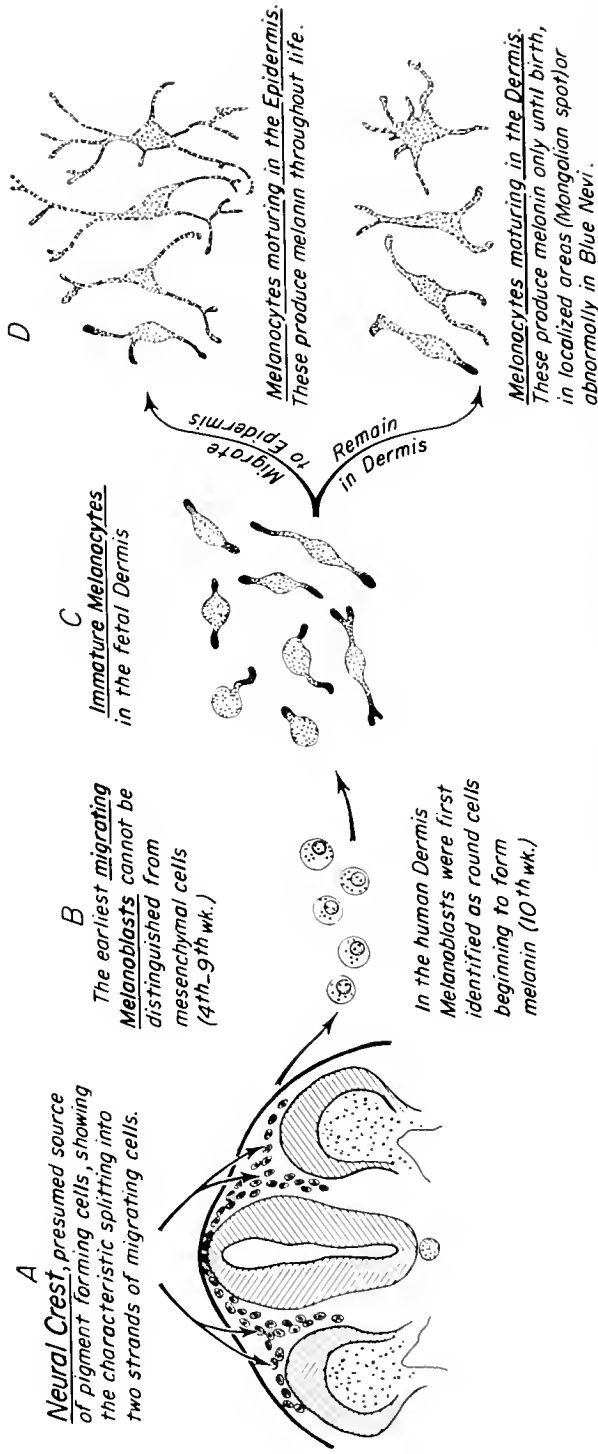
A
Neural Crest, presumed source of pigment forming cells, showing the characteristic splitting into two strands of migrating cells.

B
The earliest *migrating Melanoblasts* cannot be distinguished from mesenchymal cells (4th–9th wk.)

In the human Dermis Melanoblasts were first identified as round cells beginning to form melanin (10th wk.)

C
Immature Melanocytes in the fetal Dermis

Migrate to Epidermis

Remain in Dermis

D

Melanocytes maturing in the Epidermis. These produce melanin throughout life.

Melanocytes maturing in the Dermis. These produce melanin only until birth, in localized areas (Mongolian spot) or abnormally in Blue Nevi.

Figure 2. Schematic representation of the differentiation of melanoblasts into melanocytes and their ultimate destination in either the dermis or the epidermis. For man, the step from A–B remains problematic but has been proved to occur in mammals.

They are based on experimental evidence in mammals (Rawles, 1947). The remainder of the schematic representation is self-explanatory. The cell types shown were drawn from high power observations. The evidence for our views, of course, is mainly circumstantial since actual cell migrations and transformations cannot be observed in fixed tissues. This is a corollary of a purely morphological or histological approach.

Another question pertains to the multiplication rates of melanoblasts and melanocytes in their migratory phase. Hu, *et al.* (1957) have shown that melanocytes in cultured sheets of Negro epidermis underwent mitotic divisions. Billingham and Sparrow (quoted by Billingham and Medawar, 1953) have proved experimentally that the pigmented epidermis is "a reproductively self-sufficient system." Cell divisions occurred in both Malpighian cells and melanocytes. Masson (1948) was convinced that "melanoblasts" multiply within the basal layer of the human epidermis. He noticed features suggesting amitotic division and only once recognized a mitotic figure. Cell divisions of epidermal melanocytes have also been reported by Pinkus (1949) in a wart and by Becker Jr., Fitzpatrick, and Montgomery (1952).

We were unable to study nuclear events, since the silver impregnations of premelanin and melanin granules largely obscure them. Many cell forms of immature melanocytes in the dermis recalled Masson's observation. The manner of their possible multiplication needs further study. The numerous round melanoblasts in the scalp in late fetal periods and even at birth are also puzzling. Both cell forms may represent preparatory phases of cell division, rather than new cells derived from the neural crest (Weissenfels, 1956). Other staining methods will have to be employed to obtain a definite solution to this problem.

The main data on population densities of melanocytes in various body regions and at different times of the fetal period are briefly summarized in the conclusions of the present study.

Previous studies have shown that the average number of epidermal melanocytes per unit area is not significantly different in adult individuals of the white and Negro races. Marked numerical differences, however, do exist between individuals of either race (Szabo, 1954; Starieco and Pinkus, 1957). We found similar individual variations to exist already in the fetal period of Negroes. Our data indicate that differences in population densities of melanocytes, both regional and individual, probably become established relatively early in fetal life.

IX. Summary

Thin horizontal shavings of fetal Negro skin were impregnated with ammoniacal silver nitrate. A modified Masson technique was used and the split-skin preparations were mounted as spreads. The material consisted of 106 fetuses, varying in age from the seventh week to birth. Twenty selected skin areas were studies in each fetus. Over 500 field counts of pigment cells in the dermis and epidermis were made.

The earliest precursor stages were identified in the dermis of the tenth week of development. They appeared as round melanoblasts containing the first argentaffin granules. Still younger forms of that embryonic cell type could not be distinguished from mesenchymal cells. A gap of a few weeks remains between the development of the neural crest in man and the earliest identification of its presumptive pigmentary elements. Experimental evidence in mammals has closed that gap (Rawles).

Between the tenth and twelfth weeks, melanoblasts occur in the dermis in increasing numbers; the epidermis then contains only scattered dendritic cells. Melanoblasts differentiate into spindle-shaped, immature melanocytes with two or three short, stubby processes and increasing amounts of premelanin granules. They were identified in the dermis of all body regions, in groups or "swarms." Cell counts were highly variable.

Immature melanocytes were recognized in unstained preparations as early as the fifteenth week. Average counts of 300 to 400 per mm^2 were obtained in the thirteenth and fourteenth weeks, of 500 to 700 in the fifteenth and sixteenth weeks, and over 1,000 in the sacral region from the seventeenth to the twentieth week. From the sixth month to birth, "dermal" melanocytes were found only in the scalp, sacral area, and in the dorsum of hand and foot.

The first few epidermal melanocytes were observed in the eleventh week. Their numbers per mm^2 increased sharply between the twelfth and fourteenth weeks, indicating a period of rapid influx. No transitional forms of ordinary basal cells were seen. At first fusiform with long primary processes, the epidermal melanocytes rapidly differentiated into large dendritic cells. As early as the twelfth week they appeared in most body regions, including palm and sole. Uniform distribution patterns and high population densities became established before the end of the fourth month (800 to 1,000 melanocytes per mm^2). Their number decreased in palm and sole with the development of rete ridges (sixteenth to nineteenth weeks), but some melanocytes remain on the ridges until birth. Population densities of epidermal melanocytes remained fairly stabilized after the fifth fetal month. In the newborn Negro there were approximately 1,035 dopa-positive dendritic cells per mm^2.

Regional and individual differences in population densities of fetal

melanocytes were observed. An early cephalo-caudal gradient later dis-
appeared. It probably expressed different times of "arrival" of epidermal
melanocytes in various body regions. The earliest fully elaborated melanin
first appeared in certain head regions (third month). Gradually a dorso-
ventral gradient in population density of epidermal melanocytes emerged
and remained until birth, especially in the trunk and upper limb regions.

Individual differences in population densities were noticed as early
as the fourth fetal month.

X. Literature Cited

ADACHI, B. 1903. Hautpigment beim Menschen und bei den Affen. Zeitschr. f. Morph. u. Anthrop. 6:1-131.

BAELZ, E. 1885. Die körperlichen Eigenschaften der Japaner. Mitteilg. Deutsch. Gesellsch. f. Natur-u. Völkerkunde Ostasiens. 4:40.

BARRY, J. 1953. Recherches sur l'origine des cellules de Langerhans de l'epiderme. Biol. med. 42:293-315.

BECKER, S. W. 1927. Melanin pigmentation. A systematic study of the pigment of the human skin and upper mucous membranes, with special consideration of pigmented dendritic cells. Arch. of Dermat. and Syph. 16:259-290.

BECKER, S. W., JR., TH. B. FITZPATRICK, and H. MONTGOMERY. 1952. Human melanogenesis — Cytology and histology of pigment cells. A.M.A. Arch. of Dermat. and Syph. 65:511-523.

BECKER, S. W., JR., and A. A. ZIMMERMANN. 1955. Further studies on melanocytes and melanogenesis in the human fetus and newborn. J. Invest. Dermat. 25:103-112.

BILLINGHAM, R. E. 1948. Dendritic cells. J. Anat. 82:93-109.

———. 1949. Dendritic cells in pigmented human skin. J. Anat. 83:109-115.

——— and P. B. MEDAWAR. 1953. A study of the branched cells of the mammalian epidermis with special references to the fate of their division products. Phil. Trans. Roy. Soc. London, Series B, 237:151-171.

BLOCH, B. 1901. Preuves ataviques de la transformation des races. Bull. Soc. Anthrop. Paris, V Series, 2:618.

———. 1917. Das Problem der Pigmentbildung in der Haut. Arch. f. Dermat. u. Syph. 124:129-208.

———. 1921. Ueber die Entwicklung des Haut — und Haarpigmentes beim menschlichen Embryo und ueber das Erloeschen der Pigmentbildung im ergrauenden Haar. Arch. f. Dermat. u. Syph. 135:77-108.

DANNEEL, R., and G. CLEFFMANN. 1954. Die Einwanderung der Pigmentzellen in die Haut und die Haare bei Nagetieren. Biol. Zentralbl. 73:414-428.

EHRMANN, S. 1885. Untersuchungen ueber die Physiologie und Pathologie des Hautpigmentes. Arch. f. Dermat. u. Syph. 17:507-532.

EL BAHRAWY, A. A. 1922. Ueber den Mongolenfleck bei Europäern. Arch. f. Dermat. u. Syph. 141:171-192.

ESCHRICHT, D. F. 1849. Zool.-anat.-physiol. Untersuchungen über die nordischen Walltiere. 1:70. L. Voss, Leipzig.

GRIMM, F. 1895. Beiträge zum Studium des Pigments. Dermat. Zeitschr. 2:328-343.

HOPKINS FOX, M. 1949. Analysis of some phases of melanoblast migration in the barred Plymouth Rock embryos. Physiol. Zool. 22:1-22.

HU, F., R. E. STARICCO, H. PINKUS, and R. P. FOSNAUGH. 1957. Human melanocytes in tissue culture. J. Invest. Dermat. 28:15-32.

ISHIKAWA, N. 1924. Ueber den sogenannten Mongolenfleck bei Japanischen Foeten. Folia anat. japonica, 2:1-4.

ITO, M. 1953. Studies on Melanin. Tohoku J. Exper. Med. 55, Suppl. 1.

————. 1957. Studies on Melanin. Report II. Tohoku J. Exper. Med. 65, Suppl. V.

KATO, T. 1905. Anatomisch-histologische Studien über die sog. Kinderflecke. Mitteilg. Med. Fakult. Univ. Tokyo 6:377-396.

LANGHERHANS, P. 1868. Ueber die Nerven der Haut. Virchow's Arch. 44:325-337.

MASSON, P. 1948. Pigment cells in man, in 'Biology of Melanomas.' Spec. Publ. N. Y. Acad. Sci., 6:15-51.

MIESCHER, G. 1922. Die Chromatophoren in der Haut des Menschen. Arch. f. Dermat. u. Syph. 131:313-425.

MORISON, DR. 1889. Beiträge zur Frage von der Pigmentbildung in der Neger- haut. Monatshefte f. prakt. Dermat. 9:485-490.

PINKUS, H. 1949. Mitotic division of human dendritic melanoblasts. J. Invest. Dermat. 13:309-311.

RAWLES, M. E. 1947. Origin of pigment cells from the neural crest in the mouse embryo. Physiol. Zool. 20:248-266.

————. 1948. Origin of melanophores in development of color patterns in verte- brates. Physiol. Reviews. 28:382-408.

SCAMMON, R. E., and L. A. CALKINS. 1929. The development and growth of the external dimensions of the human body in the fetal period. University of Min- nesota Press.

STARICCO, R. J., and H. PINKUS. 1957. Quantitative and qualitative data on the pigment cells of adult human epidermis. J. Invest. Dermat. 28:33-45.

SZABO, G. 1954. The number of melanocytes in human epidermis. Brit. Med. Jour. i:1016-1020.

THOMSON, A. 1891. Note on the skin and scalp of the Negro fetus. J. Anat. Physiol. 25:282-285.

WEISSENFELS, N. 1956. Licht-phasenkontrast-und elektronenmikroskopische Un- tersuchungen über die Entstehung der Propigment-Granula in Melanoblasten- kulturen. Zeitschr. f. Zellforsch. 45:60-73.

ZIMMERMANN, A. A. 1950. The development of epidermal pigmentation in the Negro fetus. Proc. 2nd conf. on biology of norm. and atypical pigment cell growth. Zoologica, 35:10-12.

————. 1954. Die Entwicklung der Hautfarbe beim Neger vor der Geburt. Mitteilg. Thurg. Naturforsch. Gesellsch. (Switzerland) 37:33-71.

———— and TH. CORNBLEET. 1948. The development of epidermal pigmentation in the Negro fetus. J. Invest. Dermat. 11:383-392.

PLATES

Figures 3 to 26 are surface views of either full-
thickness or split-skin preparations of Negro fetuses.
All except that shown by figure 7 were impregnated
with reduced silver by a modified Masson technique.
Figure 7 represents an unstained preparation. The
magnification varies between 700 and 800 $\times$.

PLATE 1

Forearm, 12 weeks. Full-thickness skin spreads.

Early precursor stages of melanocytes in the dermis.

3 Round melanoblast, 8-10 μ diameter.

4 Early immature melanocyte forming three processes, 12–16 μ.

5 Early fusiform immature melanocyte, 20–25 μ.

6 Highly fusiform immature melanocyte 40–50 μ.
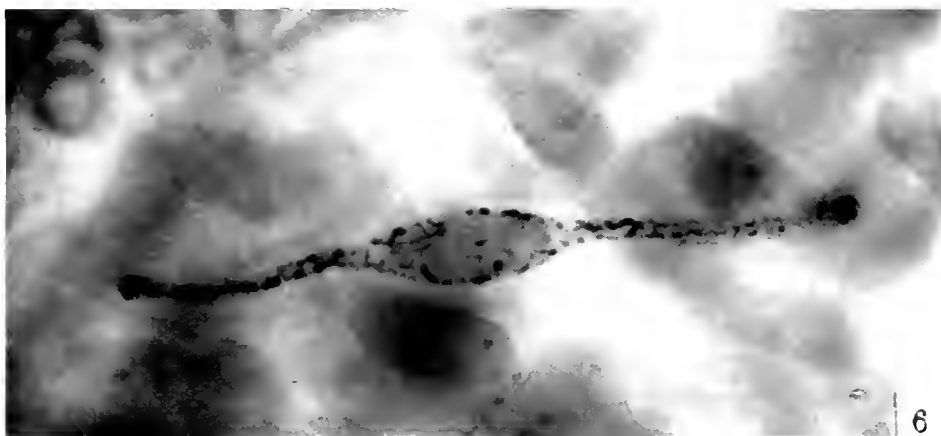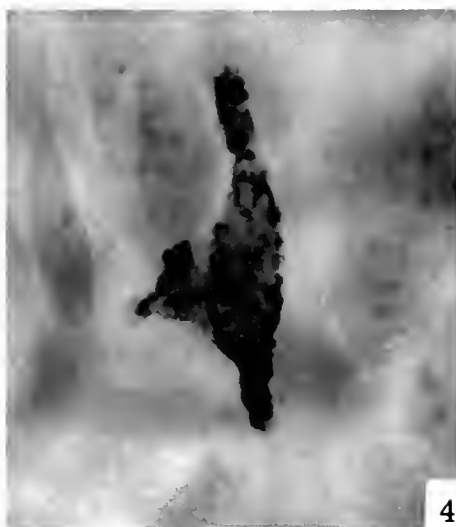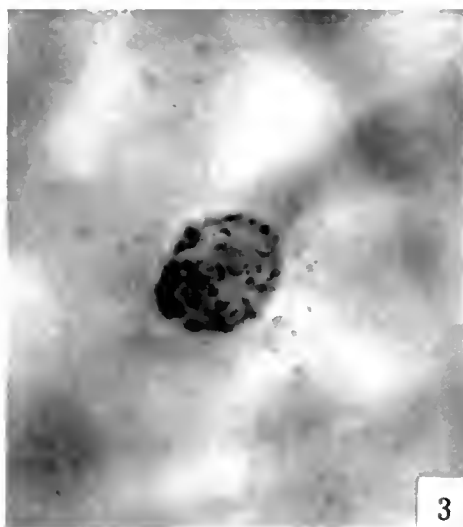
PLATE 2

7 Scalp, 15 weeks. Unstained. Two spindle-shaped, immature melanocytes in the dermis. A small blood vessel is coursing between them.

8 Dorsum of foot, 14 weeks. Fusiform melanocytes in the dermis.

9 Lumbosacral region, 13 weeks. Fusiform and early stellate forms of immature melanocytes in the dermis.
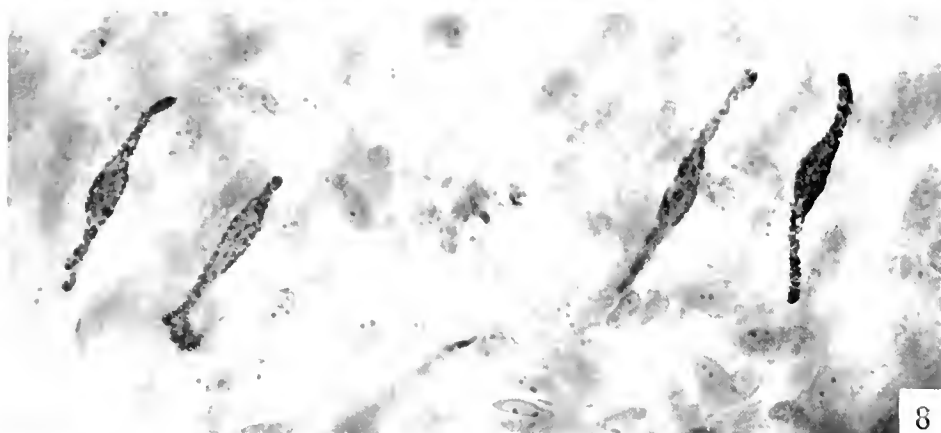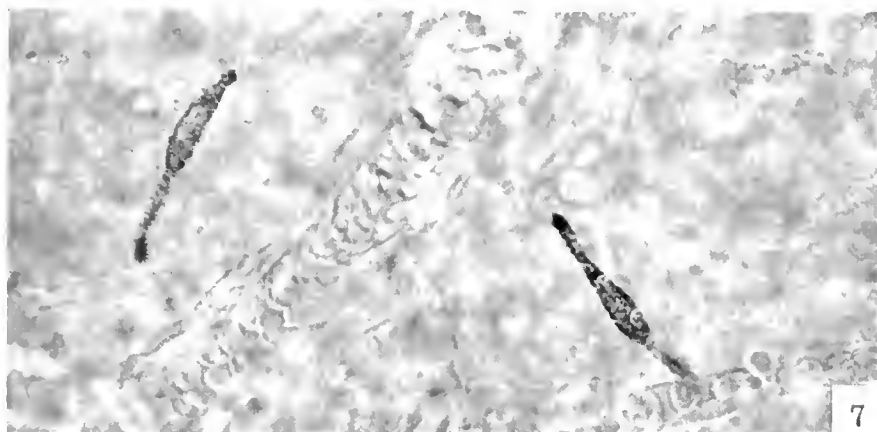
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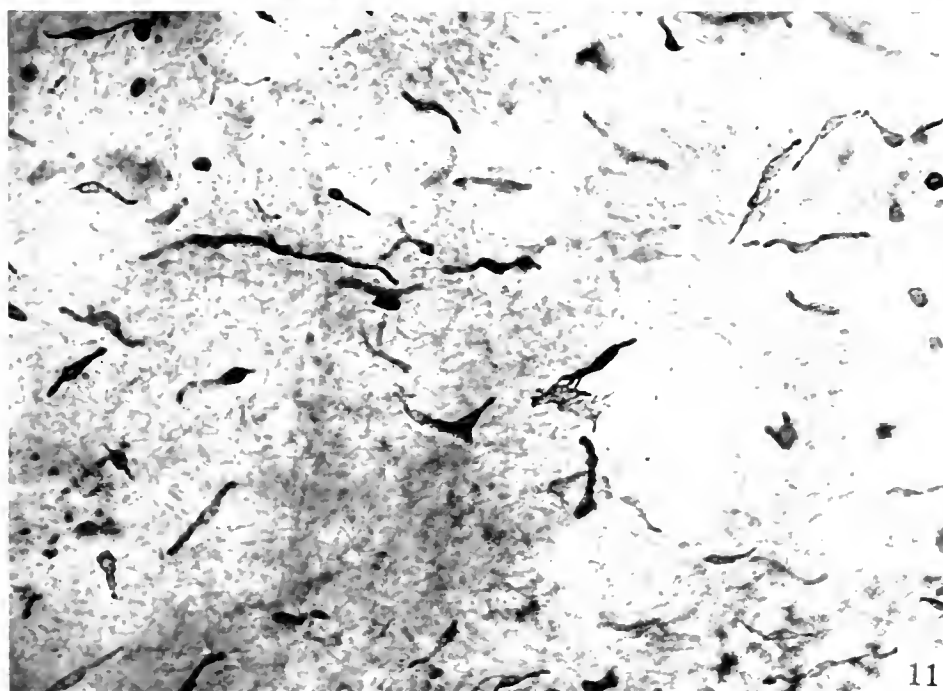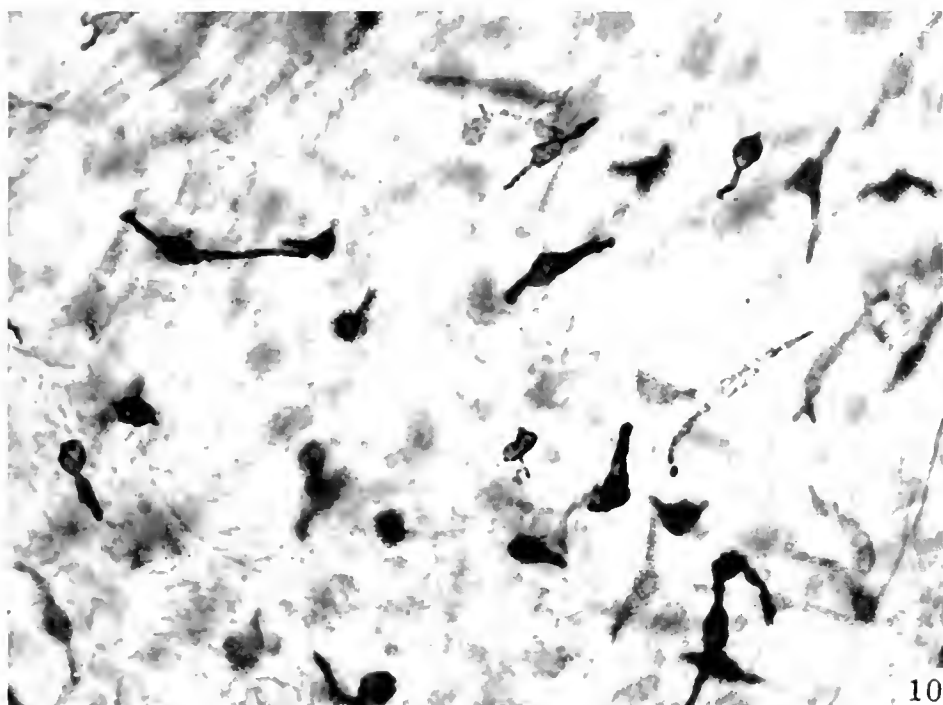
PLATE 4

12 Scalp, 18 weeks. Melanocytes adjacent to a small
   blood vessel.

13 Dorsum of hand, full-term fetus. Round, fusiform
   and stellate types of melanocytes in the dermis.

12

13

47
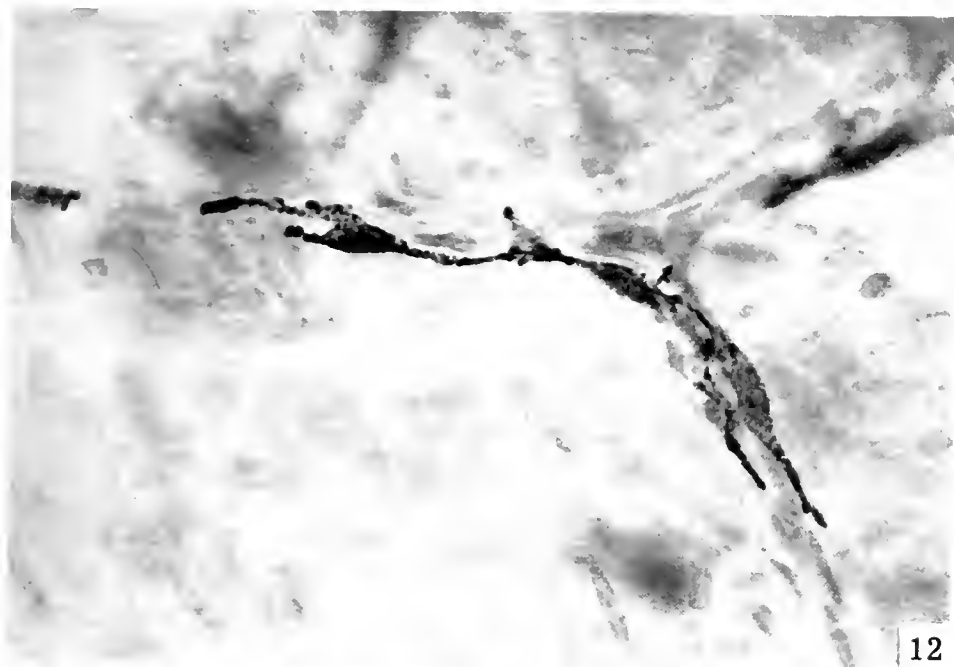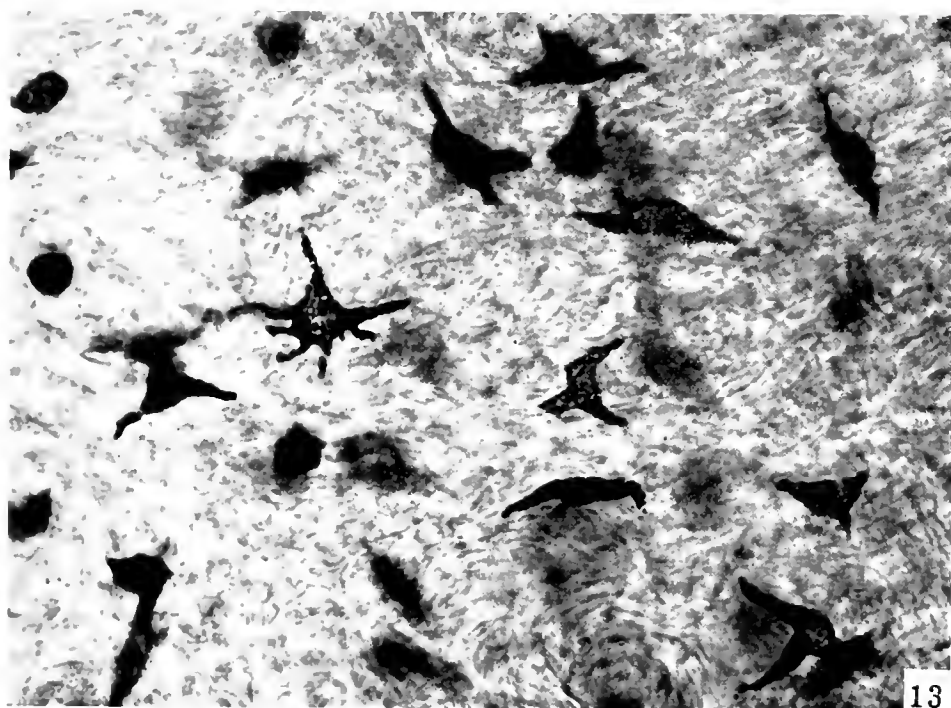
PLATE 5

14 Interscapular region, $10\frac{1}{2}$ weeks. Earliest epidermal melanocyte with three processes. Neighboring nuclei are of basal epidermal cells.

15 Sole, 12 weeks. Some of the earliest melanocytes appearing in the epidermis are highly fusiform, usually showing two long processes.

16 Nape, 12 weeks. Early epidermal melanocytes with 2-3 dendritic processes.
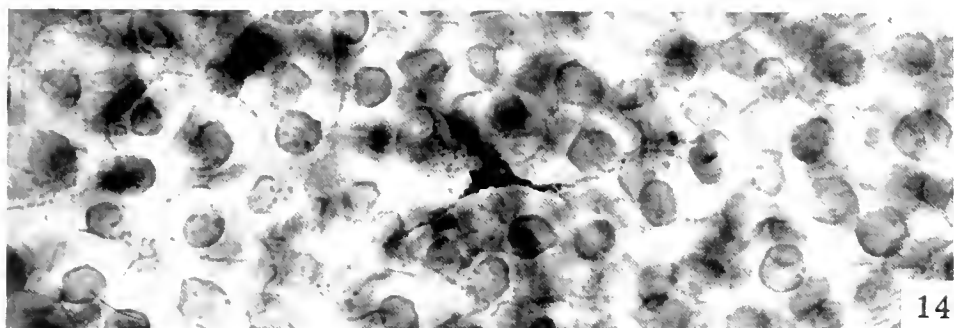
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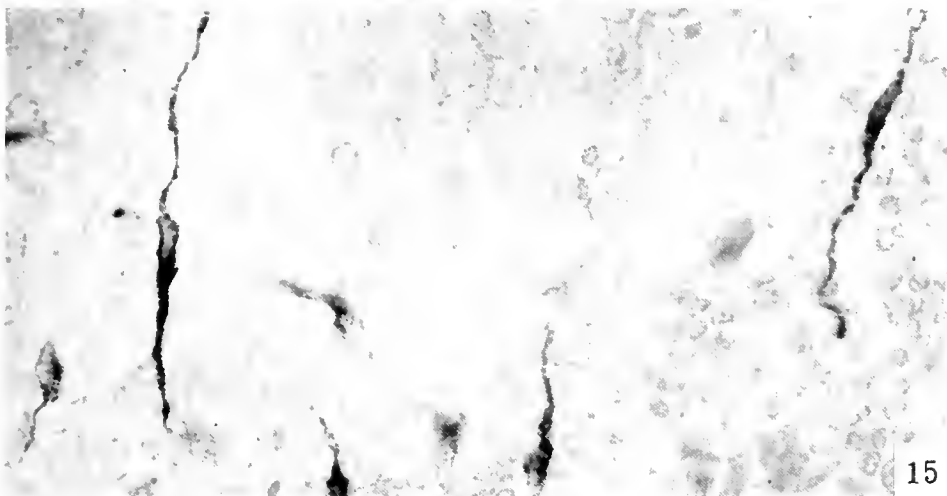
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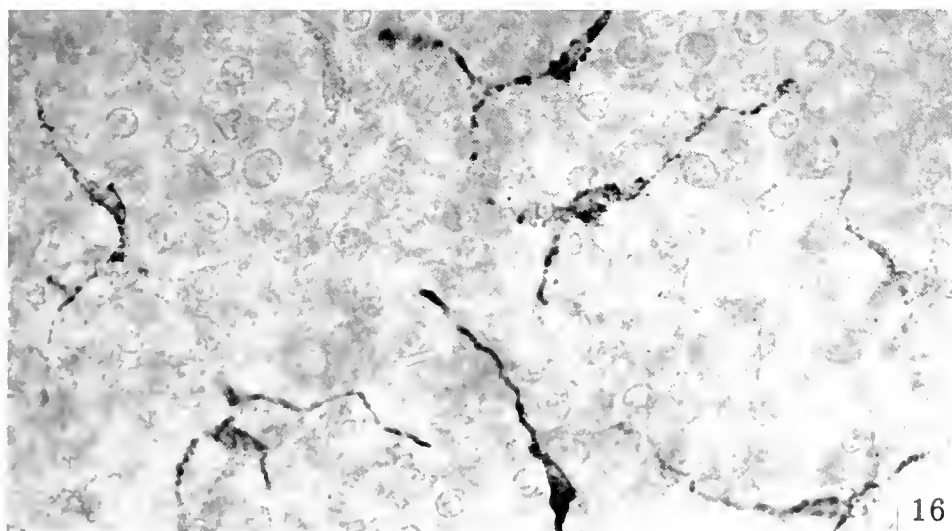
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PLATE 6

17 Scalp, 12 weeks. Earliest epidermal melanocytes in small groups, irregularly scattered through the epidermis. Cell counts are unreliable.

18 Anterior region of leg, 12 weeks. Large, heavily stained melanocytes appear here and there. The majority of epidermal melanocytes are smaller and relatively uniform in size. Neighboring nuclei represent basal cells.
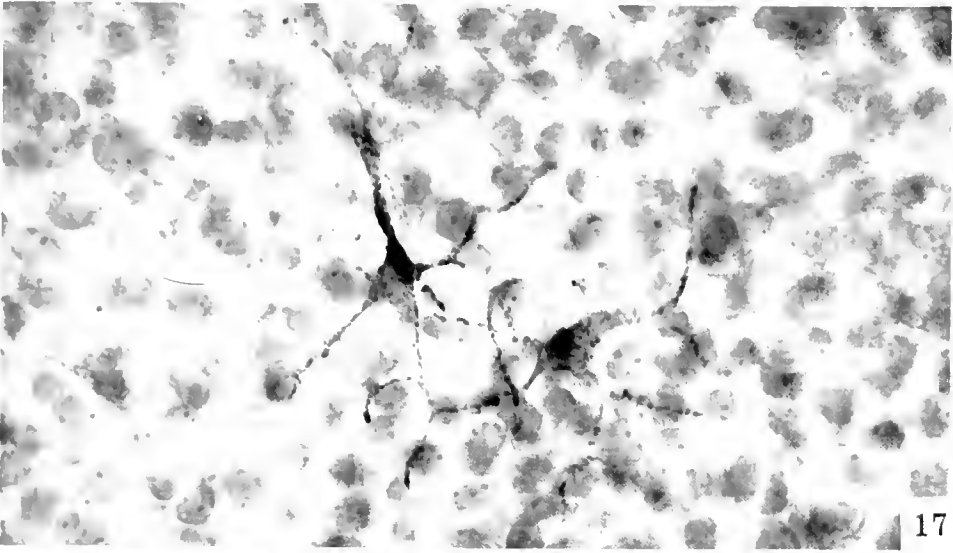
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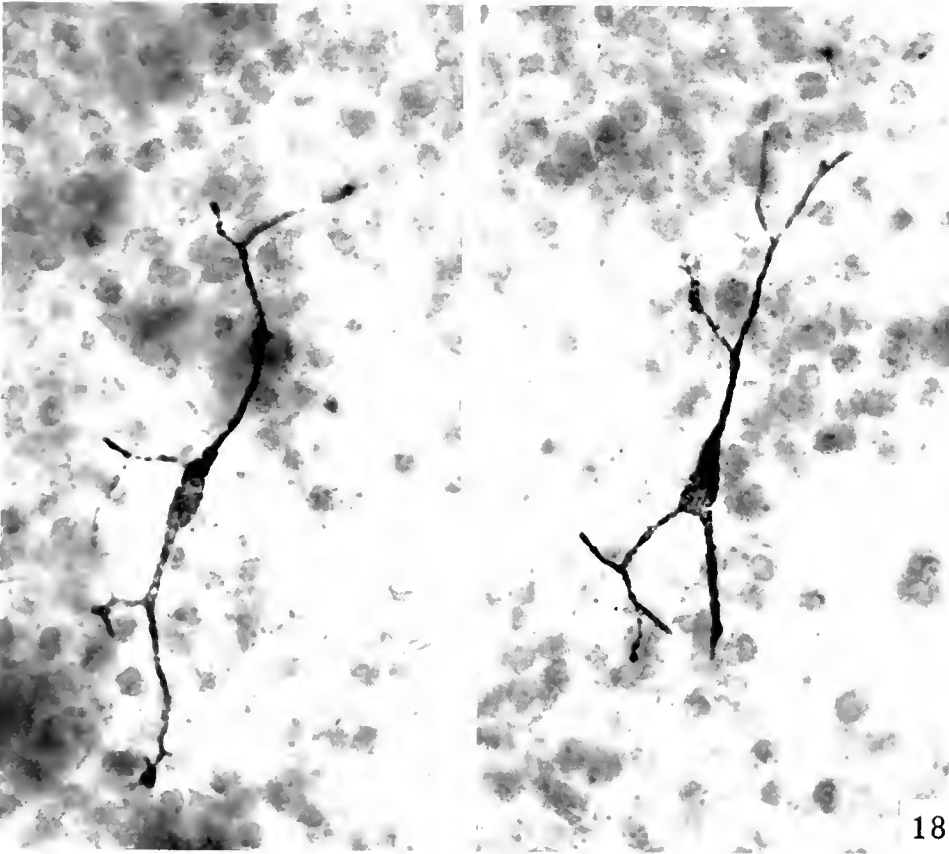
18

PLATE 7

19 Nape, $12\frac{1}{2}$ weeks. The unequal degree of silver impregnation of various melanocytes probably reflects unequal maturity or functional activity.

20 Dorsum of arm, 12 weeks. Fusiform and stellate, early dendritic cells.

19

20
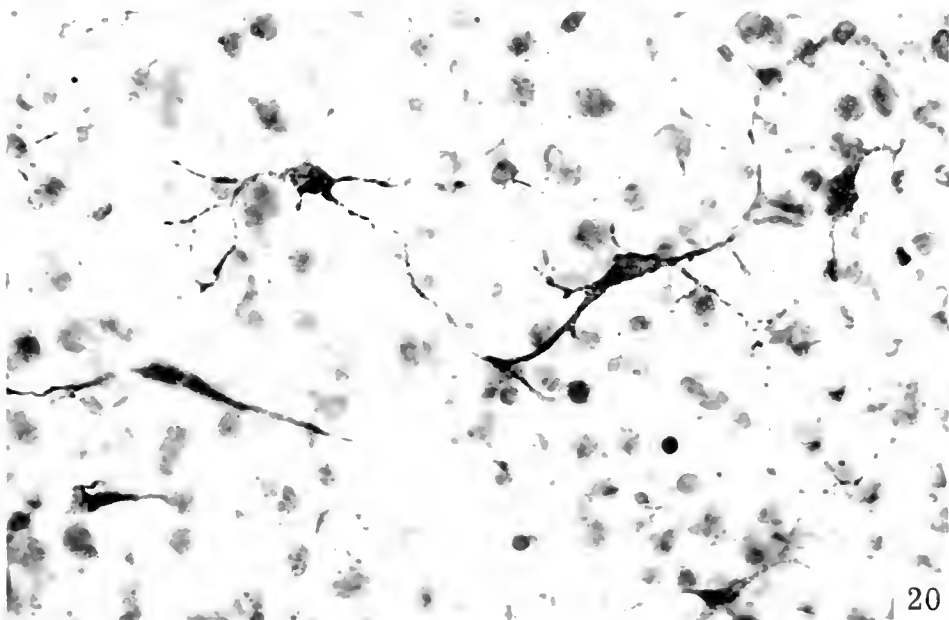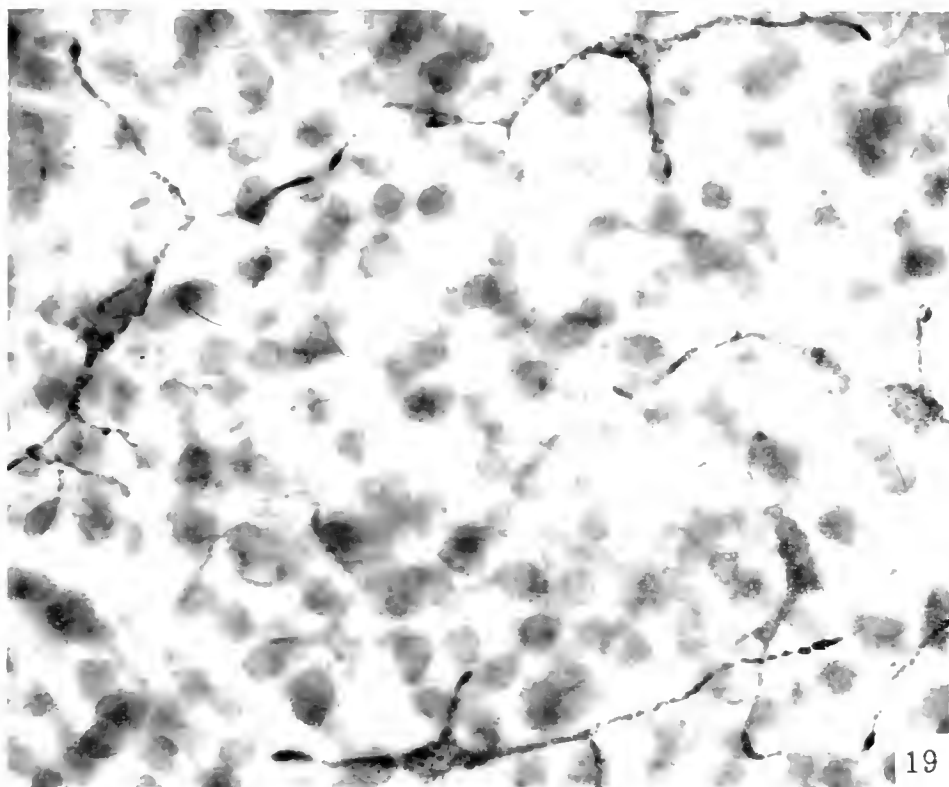
PLATE 8

Richer distribution patterns of epidermal melano-
cytes.

21 Scalp, $13\frac{1}{2}$ weeks. Regular distribution and fairly
even size of epidermal melanocytes in an interfol-
licular field.

22 Interscapular region. 15 weeks. The melanocytes
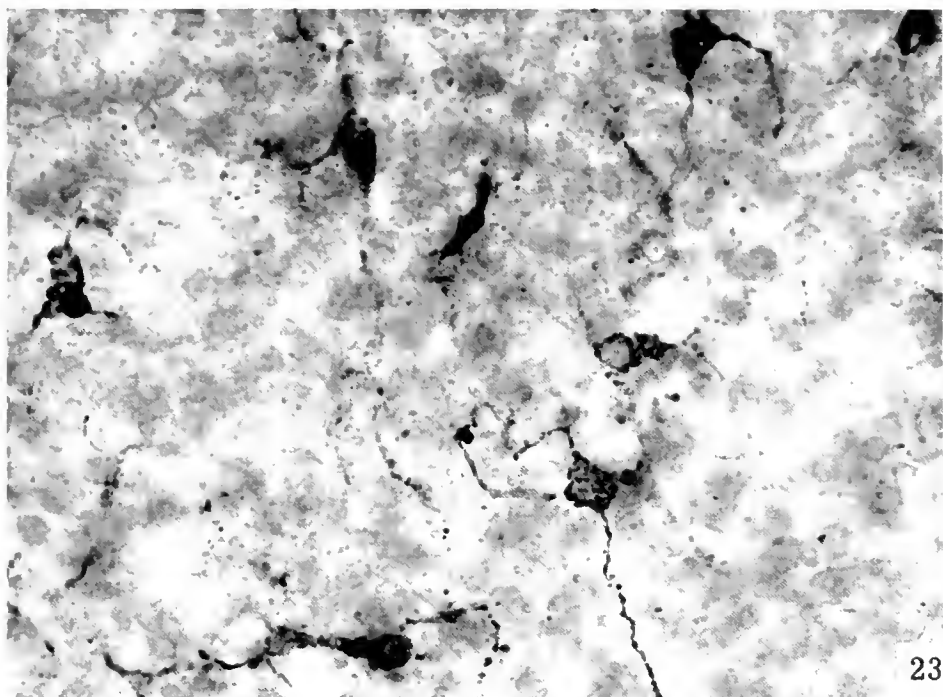appear to be oriented with their long axes running
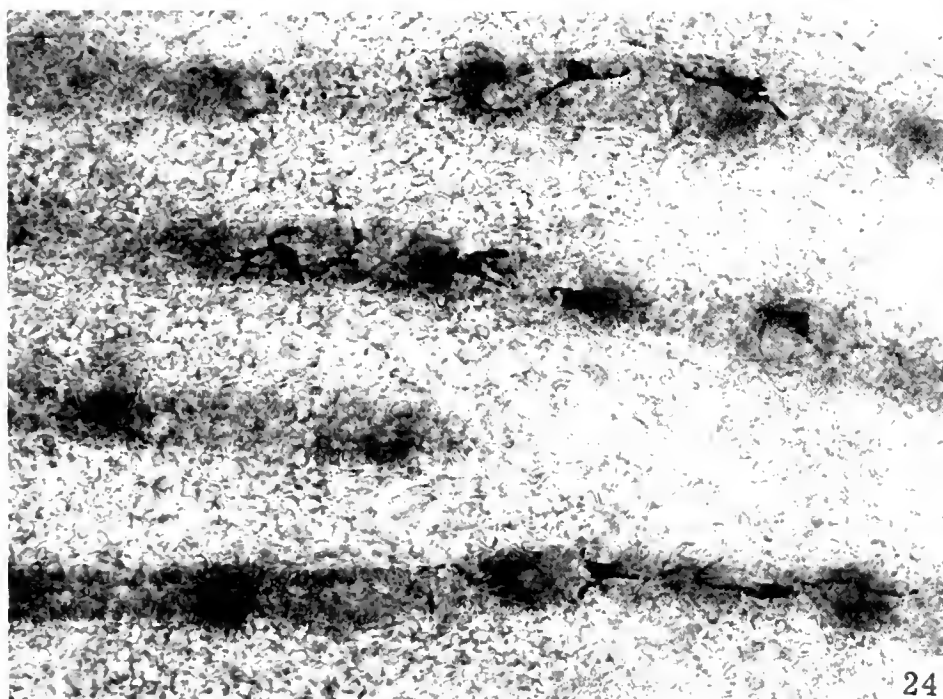parallel to each other.

21

22
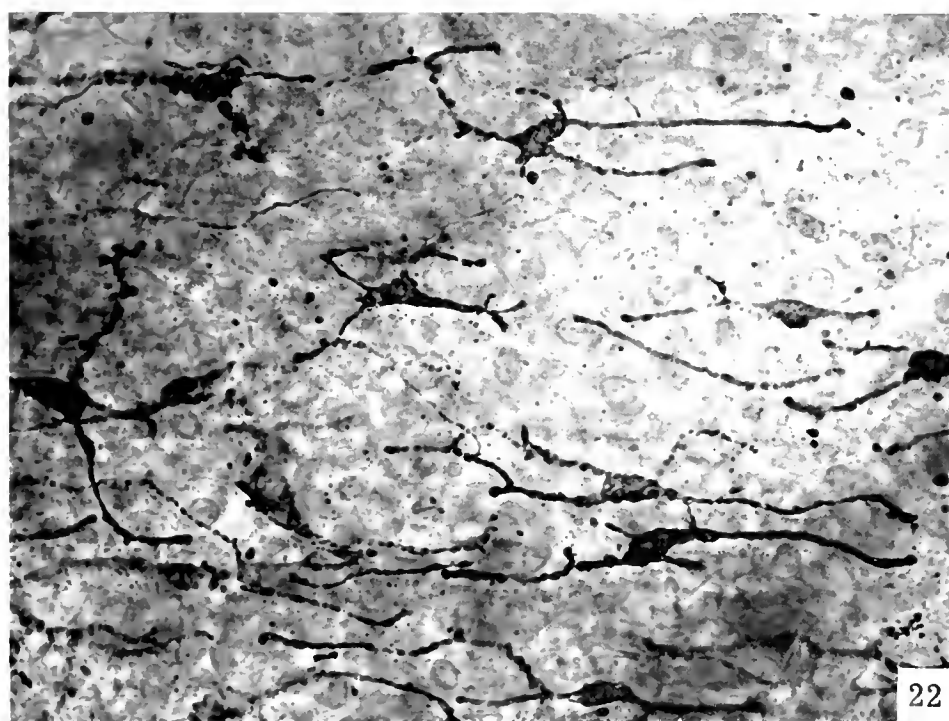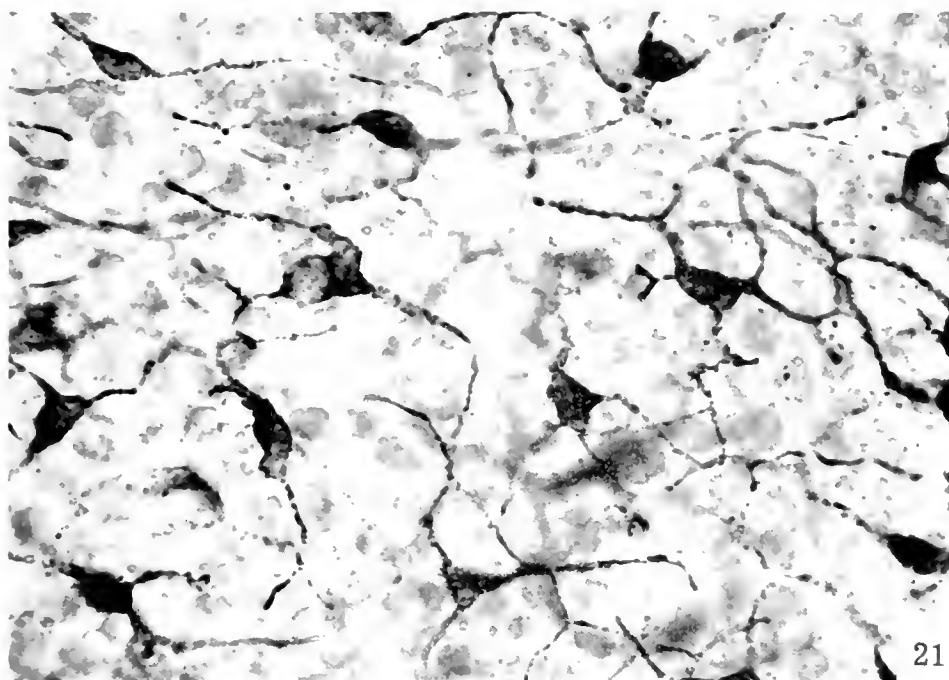
PLATE 9

23 Palm, $13\frac{1}{2}$ weeks. Fairly even distribution of epidermal melanocytes before rete ridges (epidermal crests) are developed.

24 Palm, 17 weeks. With the development of rete ridges the melanocytes come to lie on the crests. Note the developing sweat pores.
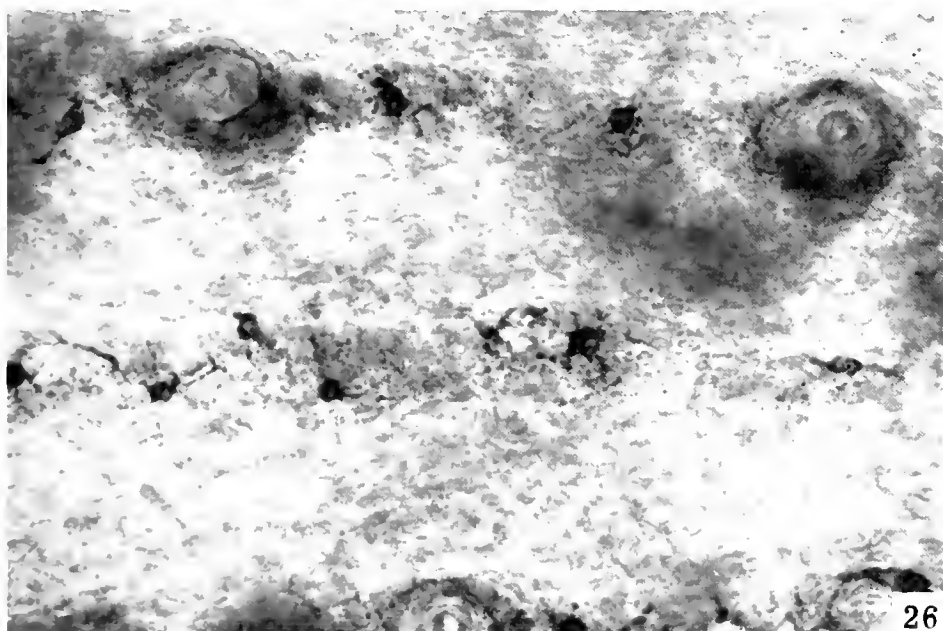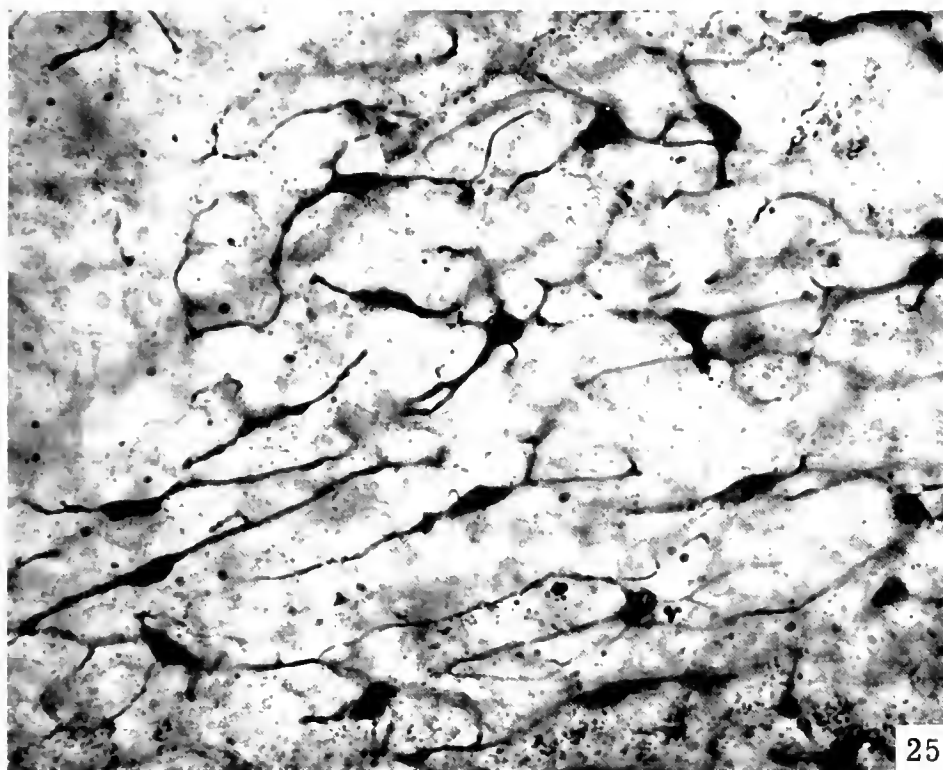
23

24

PLATE 10

25 Anterior abdominal wall, 19 weeks. Rich pattern of intraepidermal dendritic cells.

26 Palm, newborn. Epidermal melanocytes and sweat pores are recognizable on the rete ridges.

25

26

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